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# A QUANTITATIVE STUDY OF THE ANALGESIA PRODUCED BY OPIUM ALKALOIDS, INDIVIDUALLY AND IN COMBINATION WITH EACH OTHER, IN NORMAL MAN<sup>1</sup>

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## INTRODUCTION

Although the analgesia, or diminution of pain, produced by opium and its derivatives is undoubtedly the chief indication for their use, a scientific analysis of this extremely important property is still lacking, and the comparative values of the various opium alkaloids in this respect are very vaguely known. Turning to the experiences of the older observers, we are confronted with irreconcilable differences of opinion on the subject. Thus, for example, Claude Bernard (1) regarded the alkaloid narcein as possessing narcotic properties as potent as those of morphin; and the clinical observations of Sichtung (2) and Eulenburg (3) seem to sustain this view. On the other hand, von Schroeder (4), Straub (5), and other later investigators look upon that substance as entirely inert. Again, Fronmüller (6) classes the alkaloid narcotin as standing in efficiency next to morphin only, and at the same time asserts that papaverin is a more powerful analgesic than codein. Baxt (7), however, in a long dissertation on opium alkaloids, emphasizes the narcotic properties of papaverin as superior even to those of morphin, and places codein last in the list of efficient narcotics. These remarkable differences in the conclusions drawn by various observers are only to a limited extent due to impurities in the substances experimented with.

<sup>1</sup> This research has been endowed in part by a grant from the Council on Pharmacy and Chemistry of the American Medical Association.

Indeed most of the men were well trained chemists and were careful to use pure drugs. The chief explanation for the disagreement in their results lies, on the one hand, in the lack of a quantitative method of studying pain, and on the other hand, in the notoriously unreliable data gathered from the subjective symptoms and opinions of patients. Pain, being a subjective sensation, *eo ipso* precludes the possibility of direct experimentation on lower animals, and the authors cited therefore had to content themselves with clinical studies. Being unable to vary quantitatively the pain sensation, the nearest approach to a comparison of the narcotic properties of two drugs was by varying the dosage either in the same patient complaining of recurrent attacks of pain, or in two patients complaining ostensibly of exactly the same degree and kind of pain at the same time!

In connection with pharmacological studies of the principal opium alkaloids, individually and in combination with each other, carried on by one of us (M.), some of which studies have already been published (8) (9) and others of which are in progress, it was desirable to make a comparative study of these drugs in regard to their analgesic effects. For this purpose numerous observations were made on Dr. Macht and two medical students, N. B. H. and C. S. L., by the method described below.

#### METHODS OF STUDY

Through the important researches of Blix (10), von Frey (11), Head and Rivers (12), and others, the specificity of pain nerves and nerve endings, and the definite localization of pain points on the surface of the body has been firmly established. Von Frey in particular has shown the presence of definite pain points scattered more or less abundantly over different parts of the body, which respond with the same quality and intensity of pain sensation to the same stimuli. For producing his pain stimuli, that author made use of hairs of various degrees of stiffness. Following his work, various attempts to apply quantitatively graded pain stimuli by mechanical means have been made, and instruments called esthesiometers or algesimeters, such, for in-

stance, as that of Schoenborn (13), have been invented for that purpose, none of which, however, have proved satisfactory for scientific work. In the present research for the purpose of accurately and at the same time conveniently and rapidly grading the stimuli produced, we have taken advantage of the pain sensation excited by the Faradic or interrupted current of an induction coil. This method of producing and studying sensory stimuli is not a new one, and came into special prominence through the valuable contributions of Martin and his co-workers (14). The same investigator, Martin, in conjunction with Grace and McGuire (15) published practically the only pharmacological study along these lines. These authors in a study of the influence of acetphenetidin on the human sensory threshold, noted a marked lowering of the electrocutaneous sensitiveness after the administration of acetphenetidin by mouth.

The procedure employed by them was, briefly, the immersion of a finger in a liquid electrode, increasing the induced current, and noting the electrocutaneous sensibility. Although this method is somewhat different from the one employed in the present research and although the above authors were more interested in the threshold of electrocutaneous sensation than in pain, the results of their investigation encouraged us greatly in the completion of our work. This method, however, is applicable to the study of the sensations in the fingers only. In our investigation we have in every experiment made observations on four different regions of the body, thus decreasing the chances of error fourfold.

The apparatus employed by us was a large Baltzer inductorium with a vertical sliding secondary. The primary coil, 14 cm. in length, and consisting of 5 layers, 600 turns in all, of heavy insulated copper wire, 1 mm. thick, was connected in series with two dry cells of one volt each, and provided with a soft iron core. The secondary coil, 13 cm. in length and 5.5 cm. in average diameter, consists of 10,300 turns of very fine insulated copper wire and was connected with a pair of small platinum electrodes placed 2 mm. apart and conveniently fixed and insulated from each other by hard rubber. The sliding groove of the secondary

is provided with a scale graduated on one side in centimeters and on the other in the so-called Kronecker units, so frequently used by the physiologists. It was found that by varying the quantity of inductance produced in the secondary coil by moving it away or towards the primary, a point could be reached, when the sensation excited by applying the electrodes to some point on the surface of the body became distinctly painful. By numerous experiments we have found that for any group of pain points

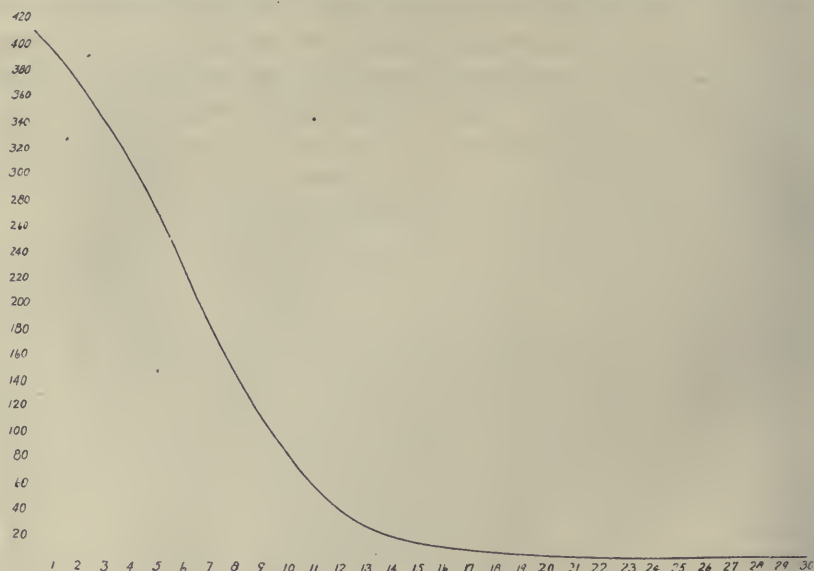


FIG. 1. CURVE OF MUTUAL INDUCTANCE.

The horizontal figures indicate positions of secondary in cm. The vertical figures indicate the mutual inductance in terms of the standard coil.

exactly the same strength of stimulus (as determined by the relative positions of the secondary and primary coils) produces pain of exactly the same intensity and quality on repeated readings and at frequent intervals, for long periods of time.

The areas most convenient for study and at the same time sufficiently sensitive to pain were found to be the skin between the thumb and index finger on the back of the hand, the tip of the nose, the tip of the tongue, and the lips. The points to which



the electrodes were applied in any one experiment were marked with ink or otherwise localized so as to be studied again whenever desired. After some practice each one of us was able easily to differentiate between changes in intensity and the quality of the pain sensation produced by moving the secondary coil as little as 0.1 cm. towards or away from the primary. In this way it was found that the threshold of pain sensation for each point

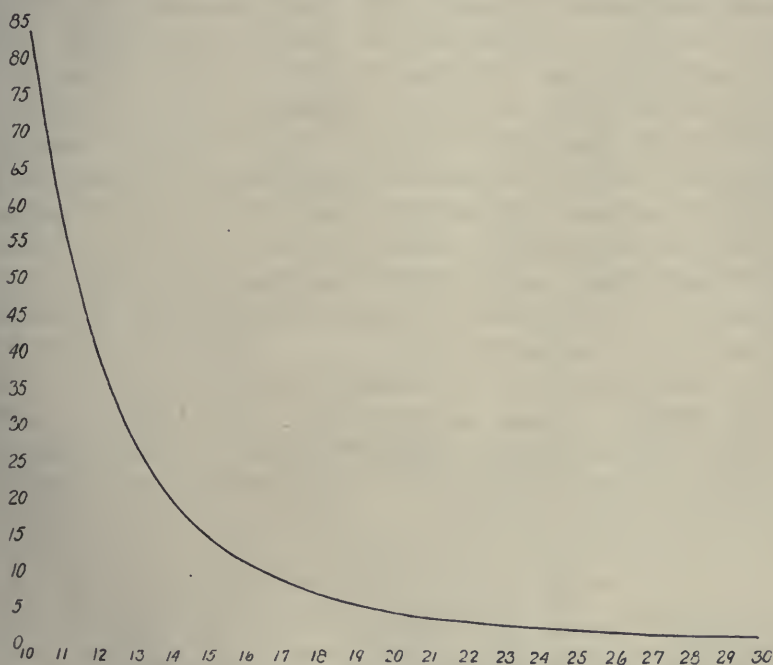


FIG. 2. Magnification of lower portion of curve of Fig. 1.

of the body is very constant. Having determined in any given experiment the strength of the stimulus required to produce pain in the different points of the body, a narcotic drug was administered by subcutaneous or intramuscular injection to the subject and after its absorption, the threshold of pain was again determined at intervals, and a fall or rise noted. The strengths of the stimuli could roughly be compared by noting the relative positions of the primary and the secondary in centimeters.

Their intensity could be still better compared by reading the Kronecker scale. The Kronecker units give us the relative galvanometer deflections for various positions of the secondary calibrated on the basis of dividing the slide into 1000 units (16). These figures are, however, arbitrary and differ for each inductorium. In order to express our results scientifically and in more definite terms, we have calculated them in absolute electrical and physical units. The difficulties in the measurement of induction shocks have been well set forth by Martin (17). We have had our apparatus calibrated in the physical laboratory of this university, and are deeply indebted to Prof. J. S. Ames and Dr. C. W. Hewlett for the work. The method of standardizing was, briefly to compare the inductance of our secondary in various positions, with the inductance of a "standard" induction coil, and plotting the results in the form of a curve. Figure 1 gives the values of our inductorium in various positions as expressed by the abscissas, in terms of the standard coil as expressed by the ordinates. Figure 2 is a magnification of the lower part of the curve. The standard induction coil was found by Dr. Hewlett to have an inductance of  $69 \cdot 10$  Henrys or 69 C. G. S. units, and so our results could be easily expressed in absolute units by multiplying the figure obtained by means of the curves, by 69.

#### REMARKS ON THE METHOD

We wish to emphasize at the outset that we are quite aware of the fact that certain subjective elements, inherent in the character of the investigation, enter into our experiments, and we have therefore taken the most painstaking precautions to eliminate as far as possible all errors arising from this source. To this end, numerous control experiments were performed.

Each experiment was carried out in the same room, under perfectly constant conditions. Readings were always taken with the subject in the same position; perfect quiet was observed; and even draughts were eliminated as possible disturbing factors. The subject was never allowed to look at the apparatus, but always sat either with eyes closed or fixed on some distant point.

The electrodes employed were of course the same throughout the experiments. The ends were blunt, so as to avoid any mechanical stimulation. The distance between the electrodes was kept fixed. Care was taken to maintain a constant wetness of the surfaces stimulated. The pressure with which the electrodes were applied was kept approximately constant, and even the direction of the application was the same. The observer or experimenter manipulating the secondary coil took care to move this secondary at a constant rate of speed.

When the drug was administered, the subject was ignorant of its nature. Furthermore, as controls, normal saline and other inactive substances were often substituted in the place of the drug without the subject's knowledge. It may be remarked in passing, that, owing to the conflicting experiences of previous observers, we were ignorant of the pharmacological action to be expected of most of the alkaloids studied, thus further eliminating any subjective bias.

#### DETERMINATION OF THE NORMAL PAIN THRESHOLD

A vital point of our investigation was the determination of the normal pain threshold for each individual in a given experiment. As the effect of the drugs administered was studied for a period of from two to six hours, it was essential to learn whether the normal readings remain constant for at least that period of time, or, if variable, their limits of variation. Martin and his co-workers by their method have noted diurnal, nocturnal and fatigue variations in the threshold of electrocutaneous sensibility. We have found in observations extending over 24 hours, very slight variations, never exceeding 0.3 cm. However, since the action of the therapeutic doses of the drugs used reached a maximum within a very short time, and most of the experiments did not last longer than six hours, the physiological variations of the normal were of little importance in our studies.

Table I gives the readings for the normal threshold for one of us, D. I. M., extending over a period of over 26 hours. In a similar way readings over periods of more than 24 hours each

TABLE I

NORMAL READINGS							SUBJECT D. I. M. 9/14/15					
Time	Hand			Tongue			Lip			Nose		
11.00 a.m.	cm.			cm.			cm.			cm.		
	10.7			11.9			14.0			13.6		
	10.7			11.9			14.1			13.6		
	10.6			11.9			14.1			13.6		
	10.7			11.9			14.1			13.6		
	10.7			11.9			14.1			13.6		
	10.7			12.0			14.1			13.5		
	10.7			12.0			14.1			13.6		
	10.7			11.9			14.0			13.6		
	10.7			11.9			14.1			13.6		
10.7			11.9			14.1			13.6			
Average . . .	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.
	235	10.7	4347	150	11.9	2760	62	14.1	1312	78	13.6	1554
11.15 a.m.	cm.			cm.			cm.			cm.		
	10.7			11.9			14.1			13.5		
	10.7			11.8			14.1			13.5		
	10.7			11.9			14.1			13.6		
	10.7			11.9			14.0			13.6		
	10.7			11.8			14.1			13.6		
	11.2			11.9			14.1			13.6		
	10.7			11.9			14.1			13.6		
	10.7			11.9			14.1			13.6		
	10.7			11.9			14.1			13.6		
10.7			11.9			14.1			13.6			
Average . . .	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.
	235	10.7	4347	150	11.9	2760	62	14.1	1312	78	13.6	1554
12.20 p.m.	cm.			cm.			cm.			cm.		
	10.7			11.8			14.1			13.6		
	10.7			11.9			14.2			13.6		
	10.9			11.8			14.1			13.5		
	10.7			11.9			14.1			13.6		
	10.7			11.9			14.1			13.6		
	10.7			11.9			14.1			13.6		
	10.7			11.9			14.0			13.5		
	10.7			11.9			14.1			13.5		
	10.6			11.8			14.1			13.6		
10.7			11.9			14.1			13.6			
Average . . .	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.
	235	10.7	4347	150	11.9	2760	62	14.1	1312	78	13.6	1554



TABLE 1—Continued

NORMAL READINGS							SUBJECT D. I. M. 9/14/15					
Time	Hand			Tongue			Lip			Nose		
1.25 p.m.	cm.			cm.			cm.			cm.		
	10.7			11.8			14.0			13.5		
	10.6			11.8			13.9			13.6		
	10.6			11.9			13.9			13.5		
	10.6			11.8			13.8			13.9		
	10.6			11.8			13.9			13.6		
	10.6			11.8			13.9			13.6		
	10.6			11.7			13.9			13.5		
	10.6			11.8			13.9			13.6		
	10.7			11.8			14.0			13.6		
10.6			11.8			13.9			13.6			
Average ...	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.
1.45*	240	10.6	4554	155	11.8	2829	68	13.9	1380	78	13.6	1554
2.20 p.m.	cm.			cm.			cm.			cm.		
	10.8			11.9			14.0			13.8		
	10.7			11.9			14.1			13.6		
	10.8			11.8			14.2			13.7		
	10.8			11.9			14.1			13.7		
	10.7			11.8			14.2			13.7		
	10.8			11.9			14.2			13.7		
	10.8			11.9			14.2			13.8		
	10.8			11.9			14.2			13.7		
	10.9			11.9			14.2			13.8		
10.8			11.9			14.2			13.7			
Average ...	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.
2.30 p.m.†	220	10.8	4209	150	11.9	2760	60	14.2	1278	74	13.7	1520
3.10 p.m.	cm.			cm.			cm.			cm.		
	10.5			11.9			14.1			13.7		
	10.5			11.8			14.1			13.7		
	10.5			11.6			14.0			13.8		
	10.4			11.7			14.1			13.7		
	10.5			11.8			14.2			13.7		
	10.6			11.8			14.1			13.7		
	10.6			11.8			14.1			13.8		
	10.5			11.8			14.1			13.7		
	10.5			11.7			14.0			13.7		
10.5			11.8			14.1			13.7			
Average ...	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.
	250	10.5	4692	155	11.8	2829	62	14.1	1312	74	13.7	1520

\* Lunch.

† Exercise (walking).

TABLE I—Continued

NORMAL READINGS						SUBJECT D. I. M. 9/14/15						
Time	Hand			Tongue			Lip			Nose		
3.20 p.m.	cm.			cm.			cm.			cm.		
	10.7			11.7			13.9			13.7		
	10.6			11.8			14.0			13.6		
	10.6			11.8			13.9			13.7		
	10.7			11.8			14.0			13.7		
	10.7			11.8			14.0			13.7		
	10.6			11.7			13.9			13.6		
	10.6			11.8			14.0			13.7		
	10.6			11.8			14.0			13.6		
	10.5			11.8			14.0			13.7		
10.6			11.8			14.0			13.7			
Average . . .	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.
	240	10.6	4554	155	11.8	2829	65	14.0	1346	74	13.7	1520
8.30 p.m.	cm.			cm.			cm.			cm.		
	10.6			11.8			14.1			13.8		
	10.7			11.7			14.2			13.7		
	10.7			11.8			14.1			13.8		
	10.7			11.8			14.1			13.7		
	10.6			11.8			14.2			13.8		
	10.6			11.8			14.1			13.6		
	10.7			11.7			14.2			13.8		
	10.7			11.8			14.1			13.7		
	10.7			11.8			14.1			13.8		
10.7			11.8			14.1			13.8			
Average . . .	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.
	235	10.7	4347	155	11.8	2829	62	14.1	1312	73	13.8	1449
9.20 p.m.	cm.			cm.			cm.			cm.		
	10.6			11.8			14.1			13.8		
	10.5			11.8			13.9			13.7		
	10.5			11.8			14.0			13.7		
	10.6			11.9			14.1			13.8		
	10.5			11.9			14.1			13.9		
	10.6			11.9			14.1			13.8		
	10.6			11.9			14.2			13.8		
	10.5			11.9			14.1			13.8		
	10.6			11.9			14.1			13.8		
10.6			11.9			14.1			13.8			
Average . . .	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.
	240	10.6	4554	150	11.9	2760	62	14.1	1312	73	13.8	1449

TABLE I—Continued

NORMAL READINGS						SUBJECT D. I. M. 9/15/15						
Time	Hand			Tongue			Lip			Nose		
9.05 a.m.	cm.			cm.			cm.			cm.		
	10.7			11.9			14.1			13.8		
	10.6			11.9			14.1			13.8		
	10.6			11.8			14.0			13.7		
	10.6			11.7			14.1			13.8		
	10.7			11.8			14.1			13.8		
	10.6			11.9			14.1			13.8		
	10.5			11.7			14.1			13.7		
	10.6			11.8			14.1			13.8		
	10.6			11.9			14.1			13.8		
10.7			11.8			14.1			13.8			
Average . . .	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.
	240	10.6	4554	155	11.8	2829	62	14.1	1312	73	13.8	1449
11.50 a.m.	cm.			cm.			cm			cm.		
	10.6			11.9			14.0			13.7		
	10.6			11.9			13.9			13.7		
	10.7			12.0			14.0			13.8		
	10.6			11.9			14.0			13.7		
	10.6			11.9			14.1			13.7		
	10.6			11.9			14.0			13.7		
	10.6			11.9			14.0			13.8		
	10.6			11.8			14.0			13.7		
	10.6			11.9			14.0			13.8		
10.7			11.9			14.0			13.7			
Average . . .	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.
	240	10.6	4554	150	11.9	2760	65	14.0	1346	74	13.7	1520
12.55 p.m.	cm.			cm			cm.			cm		
	10.6			11.9			14.0			13.8		
	10.7			11.8			14.1			13.7		
	10.7			11.9			14.0			13.8		
	10.6			11.8			14.1			13.8		
	10.6			11.9			14.1			13.8		
	10.6			11.9			14.1			13.8		
	10.6			11.9			14.0			13.8		
	10.6			11.9			14.1			13.8		
	10.7			11.9			14.1			13.8		
10.6			11.9			14.1			13.8			
Average . . .	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.
	240	10.6	4554	150	11.9	2760	62	14.1	1312	73	13.8	1449

TABLE I—Continued

NORMAL READINGS							SUBJECT D. I. M. 9/15/15					
Time	Hand			Tongue			Lip			Nose		
1.45 p.m.	<i>cm.</i>			<i>cm.</i>			<i>cm.</i>			<i>cm.</i>		
	10.6			12.0			14.0			13.7		
	10.6			11.9			14.1			13.8		
	10.7			11.9			14.1			13.7		
	10.6			12.1			14.1			13.7		
	10.7			12.0			14.0			13.8		
	10.6			12.0			14.0			13.7		
	10.7			12.0			14.0			13.7		
	10.6			11.9			13.9			13.7		
	10.6			11.9			14.0			13.7		
10.6			11.9			14.0			13.7			
Average . . .	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.
	240	10.6	4554	145	12.0	2622	65	14.0	1346	74	13.7	1520
2.20 p.m.†												
2.20 p.m.	<i>cm.</i>			<i>cm.</i>			<i>cm.</i>			<i>cm.</i>		
	10.6			11.8			13.8			13.6		
	10.6			11.8			13.8			13.6		
	10.4			11.9			13.9			13.6		
	10.6			11.9			13.8			13.5		
	10.6			11.9			13.9			13.5		
	10.6			11.9			13.9			13.6		
	10.6			11.9			13.8			13.6		
	10.5			11.8			13.8			13.6		
	10.6			11.8			13.8			13.7		
10.6			11.8			13.8			13.6			
Average . . .	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.	Kron.	cm.	C.G.S.
	240	10.6	4554	150	11.9	2760	73	13.8	1449	78	13.6	1554

† Headache (fasting).

were made on N. B. H. and C. S. L. with exactly parallel results. Owing to the great expense of printing, the tables of their normal readings are omitted.

## ACTION OF OPIUM ALKALOIDS

By the above method it was found that after establishing the normal threshold of pain for any given spot, the administration of some opium alkaloids produced a fall and that of others a rise

in the threshold of pain sensation, thus affording a quantitative method of studying analgesia. Of course by this method only cutaneous analgesia could be studied. Visceral pains and pains from other deep lying structures cannot be studied quantitatively by any method so far known.

A most important and crucial corroboration of the validity of the above method so far as it goes, was the fact that control injections of normal saline solution or of distilled water administered to the subject from time to time while under the impression that he was getting a narcotic drug, produced no change in the pain threshold.

The drugs studied by us were the six principal opium alkaloids, namely, morphin, narcotin, codein, papaverin, narcein and thebain, in the form of their salts. The specimens of the drugs used were the same as those employed by one of us (M.) in other opium researches:<sup>2</sup> that is to say, their purity was well within the limits of error of physiological experimentation. All the drugs were administered to each one of us in various doses either subcutaneously or intramuscularly.

#### THE ACTION OF MORPHIN

Morphin sulphate was used. The results of the experiments are fully expressed in the following protocols (experiments 1-7). It will be noted that very small doses of the drug (5 mgm.) produced no measurable analgesia. Slightly larger quantities, 10 mgm. (1/6 gr.), which is generally regarded as a rather moderate therapeutic dose, produced quite marked lowering of the pain threshold in two of us (exps. 1 and 2), but not in the third (H., exp. 3). In this last subject the symptoms following the narcotic—nausea, sleepiness, incoördination—were very marked, yet the pain sensation was but little affected. In order to exclude any error in the experimentation, the subject repeated the experiment, and the results were extremely interesting (exp. 7). A somewhat larger dose, 12 mgm. (1/5 gr.), was administered, and repeated observations for two and one-half hours were made. It will be noticed that the drug instead of producing an analgesia,

<sup>2</sup> See, L. E. Warren, Amer. Jour. of Pharm., 1915, lxxxvii, 439.



excited a definite hypersensibility to pain, as indicated by the rise in the threshold, reaching a maximum 1 hour and 30 minutes after the administration of the drug. The hyperexcitability could even be noticed on slamming a door or making a noise, which sounds tended to make the subject start or jump up. At the same time all the other by-effects of morphin were present to a high degree; the subject was nauseated, exhibited inco-ordination of gait and speech, and was otherwise sick. This experiment seems to us to substantiate strikingly the idiosyncrasy to morphin met with in some patients. We see occasionally persons in pain who fail to obtain relief from the drug and are rendered even more excitable by it. The above experiments prove quantitatively the existence of such a condition.

Of the other symptoms produced by morphin, we may mention here particularly the nausea, and even vomiting (exp. 1), produced by the drug. This nausea was felt also after the small doses, which had no effect on the pain sensation. It is further worthy of note, that constipation was not a marked feature in the experiments; indeed, in most of the experiments no constipation occurred. None of the morphin injections produced any pain at the point of injection.

*Experiment 1. June 30, 1915, 3.30 p.m.* Subject C. S. L. Injection of morphin sulphate, 10 mgm.

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	11.5	175	3174	16.4	29	708	14.2	60	1278	15.7	36	863
Injection of 1 cc. normal NaCl.....	11.5	175	3174	16.4	29	708	14.4	55	1173	15.5	38	915
10 mins. after injecting drug.....	9.7	320	6210	15.4	40	932	14.5	53	1156	14.8	48	1070
20 mins. after injecting drug.....	9.7	320	6210	15.5	38	915	13.3	87	1656	13.4	83	1622

*Remarks.* Pulse: before injection, 80, after, 80; blood pressure, before injection systolic, 104, diastolic, 74; after injection, systolic, 102, diastolic, 72. Dizziness, marked nausea; narcotic feeling; no constipation.

*Experiment 2. July 6, 1915, 12.15 p.m.* Subject D. I. M. Injection of morphin sulphate, 10 mgm.

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.
	cm.		units	cm.		units	cm.		units	cm.		units
Normal.....	12.1	140	2553	13.5	80	1588	15.1	44	1002	14.5	53	1156
25 mins. after injection...	10.8	220	4209	11.5	175	3174	12.5	120	2208	12.4	125	2277

*Remarks.* Pulse: before injection, 64; after injection 64. Blood pressure: before injection, systolic, 104, diastolic, 80; after injection, systolic, 104, diastolic, 80. Respiration: before, 16; after, 12 per minute. Extreme nausea; vomited three times; forced to lie down; effect felt six hours afterwards; no constipation; no pain at place of injection.

*Experiment 3. July 1, 1915, 2.15 p.m.* Subject N. B. H. Injection, morphin sulphate, 10 mgm.

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.
	cm.		units	cm.		units	cm.		units	cm.		units
Normal.....	10.8	220	4209	17.6	21	535	17.1	23	614	17.5	21	552
Injection 1 cc. nor. NaCl..	10.7	235	4347	17.6	21	535	17.0	24	622	17.5	21	552
10 mins. after injection...	10.8	220	4209	17.8	20	501	15.6	38	897	17.3	22	588
35 mins. after injection...	10.9	215	4002	17.6	21	535	14.9	48	1036	15.4	40	932

*Remarks.* Pulse: before injection, 76; after injection, 74. Systolic pressure: before injection, 124; after injection, 126. Diastolic pressure: before injection, 80; after injection, 78. Very "dopy" and dizzy. Great difficulty in concentrating and coördinating. Nauseated by riding. Very sleepy, lasting about 6 to 7 hours. No constipation.

*Experiment 4. July 16, 1915. 11.00 a.m. Subject D. I. M. Injection of morphin sulphate, 5 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	10.6	240	4554	12.9	100	1897	14.1	62	1312	15.1	44	1002
15 mins. after injection...	10.6	240	4554	12.9	100	1897	14.0	65	1346	15.0	46	1019
45 mins. after injection...	10.6	240	4554	13.0	96	1863	14.1	62	1312	14.8	48	1070
60 mins. after injection...	10.6	240	4554	13.1	94	1794	13.9	68	1380	15.1	44	1002

*Remarks.* Distinct feeling of "dopiness," though not severe; marked nausea for an hour after injection; no constipation; no pain from injection.

*Experiment 5. July 16, 1915, 1.00 p.m. Subject C. S. L. Injection of morphin sulphate, 5 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	12.3	130	2346	17.5	21	552	14.3	57	1244	16.5	28	690
15 mins. after injection...	12.3	130	2346	17.5	21	552	14.4	55	1173	16.6	27	673
30 mins. after injection...	12.4	125	2277	17.5	21	552	14.3	57	1244	16.6	27	673

*Remarks.* Nausea one hour after injection and later; headache; heaviness in feet and tingling; incoördination of speech and gait; no constipation.

*Experiment 6. July 16, 1915, 11.15 a.m. Subject N. B. H. Injection of morphin sulphate, 5 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	11.8	155	2829	19.3	15	363	15.5	38	915	18.0	18	483
10 mins. after injection...	11.8	155	2829	19.2	15	380	15.5	38	915	18.0	18	483
30 mins. after injection...	11.8	155	2829	19.2	15	380	16.0	31	778	18.0	18	483

*Remarks.* Extreme sleepiness and difficulty in concentrating and coördinating. No nausea. Maximum effect within 15 minutes of injection. Two hours later, effect nearly worn off. Three hours later, nauseated by riding on street car.



*Experiment 7. July 27, 1915, 11.00 a.m. Subject N. B. H. Injection of morphin sulphate, 12 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	10.6	240	4554	17.1	23	614	15.7	36	863	15.6	38	897
15 mins. after injection...	10.8	220	4209	17.2	23	605	15.8	36	829	15.9	33	795
30 mins. after injection...	11.0	210	3864	17.3	22	588	16.1	31	759	16.1	31	759
45 mins. after injection...	11.4	180	3312	17.6	21	535	16.5	28	690	16.3	29	725
1 hr., 15 min. after injection.....	11.7	160	2967	17.8	20	501	16.7	26	656	16.5	28	690
1 hr., 30 min. after injection.....	11.9	150	2760	18.0	18	483	16.9	25	630	16.8	26	639
2 hrs., 15 min. after injection.....	11.5	175	3174	17.5	21	552	16.0	31	778	15.9	33	795
2 hrs., 30 min. after injection.....	11.1	205	3626	17.3	22	588	15.8	36	829	15.7	36	863

*Remarks.* Pulse: before injection, 68; after injection, 72. Systolic pressure: before injection, 106; after injection, 104. Diastolic pressure: before injection, 82; after injection, 82. Decided incoördination of gait, speech, and writing. Very dizzy and *nauseated*. Dizziness and nausea greatly increased by walking about. Slept  $1\frac{1}{2}$  hours. Felt better, but still jumpy. Next day, still felt slightly nauseated, and very tired. Constipated.

#### THE ACTION OF CODEIN

This drug is treated after morphin, because next to morphin it is the most important opium alkaloid employed in practice. Codein phosphate was used, in doses of 20 and 25 mgs. The most important point to be noted in connection with this drug is its very poor analgesic power, which is not only far inferior to that of morphin, but is also less effective than papaverin, as will be described later. No nausea or other untoward symptoms followed the administration of this drug. There was no constipation noted; nor was there any pain at the site of injection. The following protocols illustrate its action.

*Experiment 8. July 13, 1915, 11.00 a.m. Subject D. I. M. Injection of codein phosphate, 20 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.
	cm.	units	units	cm.	units	units	cm.	units	units	cm.	units	units
Normal.....	12.5	120	2208	12.5	120	2208	15.2	42	966	13.5	80	1588
30 mins. after injection...	11.7	160	2967	11.6	170	3036	14.0	65	1346	12.9	100	1897
1 hr. after injection.....	12.1	140	2553	12.3	130	2346	14.1	62	1312	13.0	96	1863

*Remarks.* Pulse before injection, 72; after injection, 72; blood pressure, before injection, systolic, 108, diastolic, 72; after injection, systolic, 109, diastolic, 70; no pain from injection, no nausea, no constipation.

*Experiment 9. July 6, 1915, 11.00 a.m. Subject C. S. L. Injection of codein phosphate, 25 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.
	cm.	units	units	cm.	units	units	cm.	units	units	cm.	units	units
Normal.....	10.5	250	4692	15.9	33	795	13.5	80	1588	14.4	55	1273
15 mins. after injection...	9.5	340	6624	15.0	46	1019	13.4	83	1622	14.2	60	1278
25 mins. after injection...	9.0	375	7590	14.7	50	1104	12.8	105	1966	13.4	83	1622

*Remarks.* Slight heaviness in feet and headache; no constipation; no nausea.

*Experiment 10. July 13, 1915, 1.30 p.m. Subject N. B. H. Injection codein phosphate, 20 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.
	cm.	units	units	cm.	units	units	cm.	units	units	cm.	units	units
Normal.....	13.5	80	1588	19.2	15	380	15.5	38	915	19.1	15	397
25 mins. after injection...	12.9	100	1897	18.6	16	414	15.3	41	949	18.0	18	483

*Remarks.* Pulse: before injection, 76; after injection, 72. Systolic pressure: before injection, 110; after injection, 112. Diastolic pressure: before injection, 70; after injection, 70. No noticeable symptoms. No constipation.

## THE ACTION OF PAPAVERIN

This alkaloid, though known for some time, has been little employed in practice until recently. Of the older observations with it, those of Baxt (7) are probably the most important and interesting. This author emphasized its narcotic and analgesic properties, ranking it next to morphin. In the last few years, Pal (19) has been employing its extensively and one of us (M) recently pointed out its remarkable influence on the heart and coronary circulation (8). Its action on the respiration is also of interest (9). We experimented with papaverin hydrochloride and papaverin sulphate in doses of 40 mgm. The injections were slightly painful, but otherwise no untoward symptoms were felt. There was a slight feeling of flushing in the head and face due to vaso-dilatation produced by it, and there was a distinct fall in the blood pressure in every case. Slight constipation was noted. The analgesic properties of this drug were found to be very interesting. Its effects were certainly more powerful than those of codein, and were not inferior to those produced by 10 mgm. of morphin, except that the dose of the drug was much larger and the onset of analgesia slower and its duration shorter. The following protocols illustrate its action.

*Experiment 11. July 10, 1915, 2.20 p.m.* Subject D. I. M. Injection of papaverin sulphate, 40 mgm.

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	11.8	155	2829	14.4	55	1173	16.8	26	639	17.7	20	518
30 mins. after injection...	10.2	270	5313	12.9	100	1897	14.0	65	1346	14.6	51	1139
1 hr. after injection.....	9.7	320	6210	12.4	125	2277	13.3	87	1656	12.2	137	2484

*Remarks.* Pulse: before injection, 72; after injection, 66; blood pressure: before, systolic, 105, diastolic, 80; after, systolic, 98, diastolic, 78. Fullness in head after injection. Slight pain at place of injection; very slight feeling of heaviness and dullness in ears; no constipation.

*Experiment 12. July 8, 1915, 2.20 p.m. Subject C. S. L. Injection of papaverin hydrochloride, 40 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	10.0	285	5520	15.9	33	795	14.3	57	1244	16.2	29	742
15 mins. after injection...	8.5	425	8694	14.9	48	1036	12.8	105	1966	15.2	42	966
30 mins. after injection...	8.6	420	8349	14.2	60	1278	12.0	145	2622	15.5	38	915

*Remarks.* Pulse: before injection, 84; after injection, 84. Blood pressure, before injection, systolic, 102, diastolic, 70; after injection; systolic, 98, diastolic, 64. No untoward symptoms.

*Experiment 13. July 15, 1915, 10.40 a.m. Subject N. B. H. Injection, papaverin hydrochloride, 35 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	13.2	89	1725	19.0	15	408	16.0	31	778	19.7	13	311
20 mins. after injection...	12.5	120	2208	18.2	17	466	14.8	48	1070	18.3	17	449
40 mins. after injection...	12.3	130	2346	18.1	17	474	14.6	51	1139	18.0	18	483

*Remarks.* Pulse: before injection, 70; after injection, 64. Systolic pressure: before injection, 108; after injection, 103. No discomfort following injection, except slight soreness of arm. No constipation. No nausea.

#### THE ACTION OF NARCOTIN

The narcotic properties of this alkaloid have been and are generally regarded as very weak, although Frommüller ranked it next to morphin. Eulenburg and Baxt considered it as much weaker than codein. The chief interest about this drug is in connection with the important rôle attributed to it in certain combinations of opium alkaloids. We experimented with the hydrochloride. Narcotin being a very weak base its salts are



slightly acid, and the injections therefore caused considerable pain. No other untoward symptoms were noted, except a slight constipation following its administration. We employed doses of from 8 to 40 mgm. In regard to analgesic action the following is to be noted: Small doses of narcotin produce no analgesia, but on the contrary give rise to a slight hypersensitiveness. (Exps. 14-16.) After large doses (20 to 40 mgm.) a hypersensitive stage is first noted; this is however followed about half an hour later by a slight dulling of the pain sensation. This is well illustrated in case of N.B.H. in the protocol of Experiment 17. Similar results were noted in the other subjects, the tables being omitted for economy of space.

*Experiment 14. July 21, 1915, 11.00 a.m.* Subject D. I. M. Injection of narcotin hydrochloride, 8 mgm.

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	10.0	285	5520	11.6	170	3036	12.7	110	2070	14.3	57	1244
30 mins. after injection.	10.3	260	5106	11.7	160	2967	12.9	100	1897	14.2	60	1278
45 mins. after injection.	10.3	260	5106	11.9	150	2760	13.1	94	1794	14.7	50	1104

*Remarks.* Pain at point of injection several hours later. Slight constipation. Slight nausea.

*Experiment 15. July 21, 1915, 10.30 a.m.* Subject C. S. L. Injection of narcotin hydrochloride, 8 mgm.

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	10.1	280	5451	15.0	46	1019	13.0	96	1863	16.0	31	778
25 mins. after injection.	10.1	280	5451	15.1	44	1002	13.2	89	1725	16.4	29	708
45 mins. after injection.	10.1	280	5451	15.1	44	1002	13.2	89	1725	16.1	31	759

*Remarks.* Bad headache 5 hours after injection; pain in arm 2 hours after injection. No nausea. Slight constipation.

*Experiment 16. July 21, 1915, 11.30 a.m.* Subject N. B. H. Injection, narcotin hydrochloride, 8 mgm.

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.
	cm.		units	cm.		units	cm.		units	cm.		units
Normal.....	10.8	220	4209	17.8	20	501	16.3	29	725	19.1	15	397
20 mins. after injection.	10.9	215	4002	18.0	18	483	16.4	29	708	19.4	14	345
40 mins. after injection.	11.0	210	3864	17.9	19	492	16.4	29	708	19.5	14	328

*Remarks.* No soreness of arm or nausea 2 hours after injection. Then walked home, 3 miles. Very nauseated and dizzy on reaching home. Slight infiltration at seat of injection. No constipation.

*Experiment 17. August 10, 1915, 10.45 a.m.* Subject N. B. H. Injection, narcotin hydrochloride, 30 mgm.

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.
	cm.		units	cm.		units	cm.		units	cm.		units
Normal.....	11.0	210	3864	15.4	40	932	14.2	60	1278	14.0	65	1346
15 mins. after injection.	11.2	195	3588	15.5	38	915	14.4	55	1173	14.2	60	1278
30 mins. after injection.	10.7	235	4347	15.1	44	1002	13.9	68	1380	13.9	68	1380
1 hr. 10 mins. after injection.....	10.7	235	4347	14.8	48	1070	13.7	74	1520	13.8	73	1449
1 hr. 25 mins. after injection.....	10.9	215	4002	15.1	44	1002	13.9	68	1380	13.9	68	1380

*Remarks.* Felt dizzy; no nausea. Scarcely any pain at site of injection. No constipation.

#### THE ACTION OF NARCEIN AND THEBAIN

These two alkaloids have been generally regarded as devoid of narcotic or analgesic action. Indeed they are better known as convulsants, and thebain in its pharmacological action is closely allied to strychnin. We have experimented with the

hydrochlorides, in doses of 10 mgm. each. The injections of narcein were very painful, those of thebain much less so. As might have been expected, neither of these drugs produced any analgesic effect; on the contrary, they produced some hypersensibility to pain. The following protocols illustrate their action.

*Experiment 18. July 22, 1915, 2.00 p.m.* Subject C. S. L. Injection of narcein hydrochloride, 15 mgm.

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	11.6	170	3036	15.1	44	1002	15.1	44	1002	17.0	24	622
20 mins. after injection.	11.6	170	3036	15.2	42	966	15.2	42	966	17.0	24	622
35 mins. after injection.	11.6	170	3036	15.2	42	966	15.1	44	1002	16.9	25	630

*Remarks.* Injection very painful. Otherwise no symptoms. No nausea, no constipation.

*Experiment 19. July 26, 1915, 10.50 a.m.* Subject N. B. H. Injection, thebain hydrochloride, 10 mgm.

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	12.1	140	2553	16.4	29	708	15.1	44	1002	16.9	25	630
35 mins. after injection.	12.2	137	2484	16.5	28	690	15.2	42	966	16.9	25	630

*Remarks.* No noticeable symptoms, except tickling at site of injection. No constipation.

#### ANALYSIS OF THE ACTION OF INDIVIDUAL ALKALOIDS

On comparing the effects of the six alkaloids studied, we see that morphin and papaverin possess the most powerful analgesic properties. Of these two, considering the dosage and the

time of onset and duration of the analgesia, morphin comes first. Very small doses of morphin, however, as has been shown, produce no cutaneous analgesia; and on the other hand, full doses of papaverin, such as were employed by us (40 mgm.), are even more analgesic than moderate doses of morphin (10 mgm.). Classifying the alkaloids in the order of their narcotic efficiency, we can arrange them in order, according to the following scheme:

Morphin  $\rightarrow$  papaverin  $\rightarrow$  codein  $\rightarrow$  narcotin  $\rightarrow$  narcein  $\rightarrow$  thebain

In reference to their symptomatology, morphin is by far the most disagreeable, producing nausea and sometimes vomiting, and that even after subliminal doses. Constipation seems to follow more constantly after papaverin and narcotin than after morphin and codein. In regard to hypnosis morphin was certainly the most narcotic.

In respect to pain at the site of injection, narcein was the most painful, narcotin came next in order, and papaverin also caused slight tenderness. The other alkaloids produced little or no pain.

#### ACTION OF SOME COMBINATIONS

The difference in the pharmacological action of morphin or its salts and the galenical preparations of opium has in the last few years been attracting considerable attention. The explanation of this difference, and, in general, the relative pharmacodynamic values of the opium alkaloids individually and in combinations with each other, sometimes alluded to as the "opium problem" has been the subject of investigation on the part of a number of pharmacologists, notably of Sahli, (20) Faust, (21) and Straub (5). Of these the work of Straub and his pupils, Herrmann (22) and Caesar, (23) is perhaps the most interesting. Straub calls attention to an interesting synergism between morphin and narcotin. He speaks of narcotin as a practically inert drug, and yet, finds that when given in combination with morphin, it intensifies the properties of the latter many times. This phenomenon he calls "potentiation." Thus, for instance, Straub found that the lethal dose for mice of a mixture of equimolecular weights of



morphin and narcotin meconates, which he introduced under the name of "narcophin," is much smaller than that of morphin alone. Again, narcophin was found to influence the respiration much more efficiently than morphin and at the same time to cause less depression of the respiratory center. It was very desirable to test the behavior of a morphin-narcotin combination on pain. We have accordingly made a careful study of the effect of morphin-narcotin meconate or narcophin, and of other mixtures of morphin and narcotin, on each one of us. The results were extremely interesting and important.

#### ACTION OF MORPHIN-NARCOTIN-MECONATE

Injections of 20 mgm. of narcophin, containing proportionately not more than one-third of its weight, or about 6 mgm., of morphin meconate produced in each subject the highest degree of analgesia that we observed in our whole research. It was much higher than that produced by 10 mgm. of morphin alone, as may be seen from the protocols (exps. 20-22). The effects of this dose were still felt by us on the following day. This dose produced considerable nausea and from later investigations on toxicity by Dr. Macht is perhaps the maximal dose of narcophin which it is advisable to use.

Smaller doses of narcophin 10 mgm. each, containing about 3-4 mgm. of morphin by weight were also found to produce marked anesthesia, which in degree was little less than that produced by 10 mgm. of morphin alone. Experiment 23 will suffice as an illustration of this action on C. S. L. Exactly parallel results were found in the other subjects.

*Experiment 20. July 1, 1915, 3.30 p.m. Subject D. I. M. Injection narcophin, 20 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	10.1	280	5451	13.4	83	1622	15.8	36	829	14.5	53	1156
15 mins. after injection	9.0	375	7590	11.4	180	3312	11.8	155	2829	12.1	140	2553
30 mins. after injection	5.2	680	17664	11.1	205	3626	10.7	235	4345	11.3	190	3450

*Remarks.* Pulse before injection, 68; after injection, 64. Blood pressure: before injection, systolic, 108; diastolic, 70; after injection, systolic, 106, diastolic, 68. Respirations: before, 16, after, 12 per minute. Extreme nausea and vomiting; felt sick until next day; distinct constipation; slight pain from injection felt next day.

*Experiment 21. June 28, 1915, 3.40 p.m. Subject C. S. L. Injection of narcophin, 20 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	13.2	89	1725	15.1	44	1002	13.5	80	1588	14.5	53	1156
30 mins. after injection.	10.4	255	4899	13.2	89	1725	11.9	150	2760	12.0	145	2622

*Remarks.* Very sick; nausea and vomiting; effect felt next day; constipated. Pulse, before injection, 76; after, 68.

*Experiment 22. June 28, 1915, 3.00 p.m. Subject N. B. H. Injection, narcophin, 20 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	11.3	190	3450	18.5	17	432	18.1	18	474	17.7	20	518
30 mins. after injection	6.4	595	14076	15.6	38	897	14.5	53	1156	14.8	48	1070

*Remarks.* Pulse: before injection, 68; after injection, 7.2. Systolic pressure: before injection, 120; after injection, 118. Diastolic pressure: before injection, 80; after injection, 80. Great dizziness and nausea, especially on moving about. Could eat no dinner. Nausea lasted 7 hours. Slight constipation.

*Experiment 23. July 14, 1915, 11.20 a.m. Subject C. S. L. Injection of narcophin, 10 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	11.0	210	3864	17.5	21	552	14.7	50	1104	17.5	21	552
10 mins. after injection.	9.0	375	7590	16.8	26	639	13.8	73	1449	15.3	41	949
25 mins. after injection.	8.9	380	7797	15.4	40	932	13.0	96	1863	15.3	41	949

*Remarks.* Pulse: before injection, 72; after, 74. Blood pressure: before injection, systolic, 110, diastolic, 76; after injection, systolic 106, diastolic, 70. Slight nausea; flushing; tingling in feet; slight headache; sleepiness and inco-ordination; constipation; slight pain from injection.

*Experiment 24. July 19, 1915, 11.30 a.m. Subject D. I. M. Injection of narcophin, 5 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	11.2	195	3588	13.3	87	1656	15.6	38	897	14.9	48	1036
15 mins. after injection.	11.0	210	3864	12.9	100	1897	14.9	48	1036	14.8	48	1070
30 mins. after injection.	10.9	215	4002	12.7	110	2070	14.8	48	1070	14.0	65	1346
45 mins. after injection.	10.8	220	4209	12.5	120	2208	14.5	53	1156	13.9	68	1380

*Remarks.* Very slight narcotic feeling. Slight nausea 1 hour after injection; no constipation.

*Experiment 25. July 19, 1915, 12.00 m. Subject C. S. L. Injection of narcophin, 5 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	9.6	335	6384	16.7	26	656	14.2	60	1278	15.7	36	863
15 mins. after injection.	9.0	375	7590	15.4	40	932	13.3	87	1656	15.1	44	1002
25 mins. after injection.	8.9	380	7797	15.3	41	949	13.4	83	1622	15.1	44	1002

*Remarks.* Slight narcosis; slight nausea; no constipation.

*Experiment 26. July 19, 1915, 12.30 p.m.* Subject N. B. H. Injection, narcophin, 5 mgm.

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	12.2	137	2484	20.1	12	276	17.1	23	614	20.2	12	242
20 mins. after injection.	11.3	190	3450	19.3	15	363	16.5	28	690	19.5	14	328
30 mins. after injection.	11.6	170	3036	19.3	15	363	16.3	29	725	19.3	15	363

*Remarks.* Slight dizziness and heaviness of head. No constipation.

*Experiment 27. August 10, 1915, 12.05 p.m.* Subject D. I. M. Injection, narcotin hydrochloride, 8 mgm. plus morphin sulph. 5 mgm.

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	10.4	255	4899	10.8	220	4209	12.0	145	2622	13.0	96	1863
15 mins. after injection.	9.8	310	5934	10.2	270	5313	11.7	160	2967	11.9	150	2760
30 mins. after injection.	9.9	295	5796	10.1	280	5451	11.5	175	3174	11.7	160	2967
1 hr. after injection.....	9.4	350	6831	10.0	285	5520	11.2	195	3588	11.4	180	3312

*Remarks.* 15 minutes after injection: distinctly narcotic sensation. No nausea.



*Experiment 28. August 10, 1915, 11.40 a.m. Subject C. S. L.*  
Injection, narcotin hydrochloride, 30 mgm.; morphin sulphate, 3 mgm.

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	9.8	310	5934	12.2	137	2484	12.4	125	2277	15.7	36	863
Narcotin HCl 30 mgs.												
30 mins. after injection.	10.0	285	5520	12.6	115	2139	12.7	110	2070	15.8	36	829
50 mins. after injection.	10.1	280	5451	12.6	115	2139	12.5	120	2208	15.7	36	863
1 hr. 5 mins. after in- jection.....	10.0	285	5520	12.7	110	2070	12.8	105	1966	15.7	36	863
1 hr. 20 mins. after in- jection.....	10.1	280	5451	13.1	94	1794	12.8	105	1966	15.7	36	863
Morphin sulph. 3 mgm...												
10 mins. after injection.	10.1	280	5451	12.7	110	2070	12.6	115	2139	15.3	41	949
25 mins. after injection.	9.6	335	6384	12.7	125	2277	12.3	130	2346	15.1	44	1002
35 mins. after injection.	9.6	335	6384	12.1	140	2553	12.4	125	2277	15.6	38	897

Reducing the dosage still further we found that even 5 mgm. of narcophin, containing less than 2 mgm. of morphin and a little more than 3 mgm. of narcotin, respectively, also produced analgesia (exp. 24-26). Inasmuch as we have already shown that small doses of morphin (5 mgm.) and narcotin (10 mgm.) given alone cause no lowering of the pain threshold, these experiments substantiate conclusively and strikingly Straub's views on the synergism of morphin and narcotin. To confirm this interesting phenomenon further and to prove that this peculiar synergism is not due to the meconic acid present in narcophin, one of us repeated the experiment by taking a mixture of morphin sulphate (5 mgm.) and narcotin hydrochloride (8 mgm.). The results were in perfect agreement with the other observations (exp. 27).

To make the proof more complete, other experiments were made in which the subjects were first injected with narcotin hydrochloride and its effects studied for about an hour and a half; a minute dose of morphin sulphate (3 mgm.) was then ad-



ministered, and it was surprising to note that even with such a small dose, the synergism could be distinctly observed and measured (exp. 28).

In connection with this remarkable synergistic phenomenon, it was interesting to note that one of us (H), who showed an idiosyncrasy for morphin and was rendered hypersensitive by it, showed no such idiosyncrasy after narcophin. The practical application of such an observation is quite obvious.

In regard to other symptoms following the administration of narcophin, we may note from the protocols that, except after the largest doses there was on the whole much less nausea produced than after an equivalent amount of morphin alone. Constipation followed the administration of narcophin much more frequently than that of morphin. There was but slight pain from the drug at the site of injection.

#### THE ACTION OF TOTAL OPIUM ALKALOIDS

Following our noteworthy experiences with narcophin, it was desirable to test the action of some other combination of opium alkaloids. We, therefore, thought it profitable to study the effect of pantopon, or pantopium, Sahli's combination of the total alkaloids of opium. The following protocols illustrate the action of 10 mgm. of pantopon (exp. 31-33). It will be seen that 10 mgm. of the total alkaloids of opium containing not more than half its weight of anhydrous morphin produced far more analgesia than 5 mgm. of morphin alone. The nausea, on the contrary, was in each case less. There was no pain at the site of injection, and slight constipation was noted after the drug.

*Experiment 29. July 22, 1915, 11.30 a.m. Subject D. I. M. Injection of pantopon, 10 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm.			cm.		
Normal.....	10.4	255	4899	12.7	110	2070	14.5	53	1156	13.4	83	1622
15 mins. after injection.	9.6	335	6384	11.6	170	3036	13.7	74	1520	12.4	125	2277
30 mins. after injection.	9.5	340	6624	11.2	195	3588	13.2	89	1725	12.0	145	2622
1 hr. 30 mins. after injection.....	9.9	295	5796	12.0	145	2622	13.9	68	1380	13.1	94	1794

*Remarks.* Deep narcosis; inability to concentrate mind; incoördination; heaviness in limbs; no nausea or dizziness; no pain from injection; slight constipation.

*Experiment 30. July 23, 1915, 11.00 a.m. Subject C. S. L. Injection of pantopon, 10 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units	Position of secondary	Kronecker units	C. G. S. units
	cm.			cm.			cm			cm.		
Normal.....	11.1	205	3626	14.3	57	1244	14.1	62	1312	15.1	44	1002
30 mins. after injection.	10.2	270	5313	13.2	89	1725	13.1	94	1794	14.5	53	1156
45 mins. after injection.	10.0	285	5520	13.0	96	1863	13.0	96	1863	14.3	57	1244
1 hr. after injection.....	10.1	280	5451	13.5	80	1588	13.4	83	1622	14.8	48	1070

*Remarks.* Sleepiness; no nausea; no pain at point of injection; slight constipation.

*Experiment 31. July 22, 1915, 10.45 a.m. Subject N. B. H. Injection, pantopon, 10 mgm.*

	HAND			TONGUE			LIP			NOSE		
	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.	Position of secondary	Kronecker	C. G. S.
	cm.	units	units	cm.	units	units	cm.	units	units	cm.	units	units
Normal.....	11.6	170	3036	16.1	31	759	17.0	24	622	15.7	36	863
25 mins. after injection.	10.1	280	5451	15.3	41	949	15.8	36	829	14.7	50	1104
35 mins. after injection.	10.1	280	5451	14.6	51	1139	15.5	38	915	14.7	50	1104
45 mins. after injection.	10.1	280	5451	15.0	46	1019	15.9	33	795	14.9	48	1036
3 hrs. 40 mins. after injection.....	10.5	250	4692	15.6	38	897	16.2	29	742	15.2	42	966

*Remarks.* Slight nausea, 5 minutes after injection. Better in a short time. Extreme dizziness on going down stairs. Very sleepy: similar to morphine. Slight constipation.

DISCUSSION

In order to facilitate the comparison of the analgesic action of the various alkaloids and combinations of alkaloids studied, we have prepared comparative tables showing the normal pain threshold and the point of highest analgesia or hyperalgesia following the injection of the drugs, in absolute physical units for each of the observers. (Tables II-IV.)

TABLE II  
*Showing maximal effects of the various drugs used for D. I. M.*

	TIME OF READING	MORPH. SULPH. 10 MGM.	MORPH. SULPH. 5 MGM.	PAPAV. SULPH. 40 MGM.	CODEIN PHOS. 20 MGM.	NARCOTIN HCl 20 MGM.	NARCOTIN HCl 8 MGM.	NARCEIN HCl 10 MGM.	THEBAIN HCl 10 MGM.	NARCOPHIN 20 MCM.	NARCOPHIN 10 MCM.	NARCOPHIN 5 MCM.	PANTOPON 10 MCM.
		C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units
Hand.....	Before injection ...	2553	4554	2829	2208	2484	5520	6624	7038	5451	3174	3588	4899
	After injection ...	4209	4554	6210	2967	3036	5106	6210	7038	17664	5106	4209	6624
Tongue...	Before injection ...	1588	1897	1173	2208	2277	3036	2760	2622	1622	1725	1656	2070
	After injection ...	3174	1794	2277	3036	2553	2760	2622	2553	3626	2829	2208	3588
Lip.....	Before injection ...	1002	1312	639	966	1104	2070	1312	2139	829	932	897	1156
	After injection ...	2208	1380	1656	1346	1588	1794	1312	2070	4345	1139	1156	1725
Nose.....	Before injection ...	1156	1002	518	1588	1244	1244	1622	1794	1156	1156	1036	1622
	After injection ...	2277	1070	2484	1897	1656	1104	1520	1794	3450	2070	1380	2622

TABLE III

*Showing maximal effects of the various drugs used for N. B. H.*

	TIME OF READING	MORPH. SULPH. 10 MGM.	MORPH. SULPH. 5 MGM.	PAPAV. HCl 35 MGM.	CODIEN PHOS. 20 MGM.	NARCOTIN HCl 30 MGM.	NARCOTIN HCl 8 MGM.	NARCEIN HCl 10 MGM.	THEBAIN HCl 10 MGM.	NARCOPHIN 20 MGM.	NARCOPHIN 10 MGM.	NARCOPHIN 5 MGM.	PANTOPON 10 MGM.
		C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units
Hand.....	Before injection...	4209	2829	1725	1588	3864	4209	2070	2553	3450	1966	2484	3036
	After injection....	4002	2829	2346	1897	4347	3864	1897	2484	14076	4209	3450	5451
Tongue...	Before injection...	535	363	408	380	932	501	552	708	432	492	276	759
	After injection....	535	380	474	414	1070	483	535	690	897	966	363	1139
Lip.....	Before injection...	897	915	778	915	1278	725	656	1002	474	501	614	622
	After injection....	1036	778	1139	949	1520	708	656	966	1156	829	725	915
Nose.....	Before injection...	552	483	311	397	1346	397	432	630	518	605	242	863
	After injection....	932	483	483	483	1449	328	414	630	1010	1070	363	1104

TABLE IV

*Showing maximal effects of the various drugs used for C. S. L.*

	TIME OF READING	MORPH. SULPH. 10 MGM.	MORPH. SULPH. 5 MGM.	PAPAV. HCl 40 MGM.	CODIEN PHOS. 25 MGM.	NARCOTIN HCl 40 MGM.	NARCOTIN HCl 8 MGM.	NARCEIN HCl 15 MGM.	THEBAIN HCl 8 MGM.	NARCOPHIN 20 MGM.	NARCOPHIN 10 MGM.	NARCOPHIN 5 MGM.	PANTOPON 10 MGM.
		C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units	C. G. S. units
Hand.....	Before injection...	3174	2346	5520	4692	4692	5451	3036	3174	1725	3864	6384	3626
	After injection....	6210	2277	8694	7590	5313	5451	3036	2967	4899	7797	7797	5520
Tongue...	Before injection...	708	552	795	795	1622	1019	1002	1002	1002	552	656	1244
	After injection....	932	552	1278	1104	1863	1002	966	1002	1725	932	949	1863
Lip.....	Before injection...	1278	1244	1244	1588	1656	1863	1002	1278	1588	1104	1278	1312
	After injection....	1656	1173	2622	1966	1966	1725	966	1002	2760	1863	1656	1863
Nose.....	Before injection...	863	690	742	1173	1312	778	622	915	1156	552	853	1002
	After injection....	1622	673	966	1622	1554	708	630	630(?)	2622	949	1002	1244

As we have already seen, the efficiency of the individual alkaloids in this respect is indicated by the following order:  
 Morphin → papaverin → codein → narcotin → narcein → thebain

It is worthy of note that papaverin shows more analgesic properties than is generally supposed, and that narcotin is



comparatively inert when given alone. Morphin is remarkable for the extreme nausea and even vomiting which follow its use, the same occurring even when it exerts no analgesic effect. It is further interesting to note that it produced hypersensitiveness to pain instead of analgesia in one of us. The combination of morphin and narcotin exhibits a remarkable form of synergism, inasmuch as it produces distinct analgesia in small doses, a result which cannot be explained as the arithmetical summation of the effects of its component parts. What the explanation of this phenomenon may be, is not clear. It is, however, interesting to note that morphin is a representative of the class of opium alkaloids belonging to the pyridin-phenanthrene group.

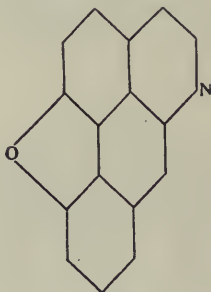


FIG. 3. Pyridin-phenanthrene group.

Narcotin, on the other hand, is characterized by the benzyl-isoquinoline group.

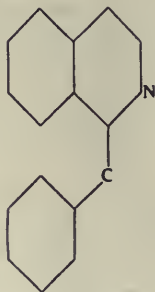


FIG. 4. Benzyl-isoquinoline group.



In narcophin we, therefore, have a combination of members belonging to two distinct chemical groups. Such a combination as has been pointed out by Bürgi, (24) is apt to lead to a potentiation rather than to a summation of the properties of the individual drugs. That author experimenting with various combinations of drugs, has advanced an hypothesis that the effect of combining two drugs belonging to the same chemical series will result in an addition of their individual effects; combinations, on the other hand, of drugs belonging to different chemical series, are prone to potentiate each other.

In spite of the subjective elements inherent in the nature of our investigation we venture to regard the analgesic properties of the various alkaloids studied as essentially correct in the order given above. We do so on account of the clear cut results and corroboratory evidence of numerous control experiments. As a matter of fact, the subjective elements in some of our experiments, if playing any rôle, would tend to produce results different from those obtained. Thus, for instance, in the case of papaverin, none of us had any idea of the marked analgesia produced by it, and, indeed, we expected it to exhibit but very mild lowering of pain threshold. Again, in the case of one of us (H), of whose idiosyncrasy to morphin we were ignorant, and in which we expected to find a marked analgesia, the hypersensitiveness to the drug, as indicated by the readings, was a very striking corroboration of the validity of our experiments. In further support of the validity of our observations, it may be well to note that the diurnal variations in the sensory threshold noted by Grabfield and Martin, if anything, tend to strengthen our conclusions. These authors found a slight rise in the sensory threshold from 9.30 to 11.30 a.m. Now it so happens that some of our experiments in which the lowering of the pain threshold was most striking were performed between these hours, and hence the lowering of the pain threshold could only be referred to the action of the drugs used.

## SUMMARY

1. A convenient method for studying cutaneous pain sensation in man quantitatively and expressing the results in absolute physical units has been described.

2. The principal opium alkaloids in respect to their analgesic power can be arranged in the following order: Morphin, papaverin, codein, narcotin, narcein, and thebain.

3. It has been found that a combination of morphin and narcotin, and also a combination of the total opium alkaloids is much more effective than the quantity of morphin they contain would be if given alone.

4. It has been further found that in the case of one of us, showing an idiosyncrasy for morphin, that alkaloid produced a heightening instead of a lowering of the threshold of pain, which phenomenon, however, disappeared on administering morphin in conjunction with narcotin.

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## SOME OBSERVATIONS ON THE ELIMINATION OF HEXAMETHYLENETETRAMINE (UROTROPIN)

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Hexamethylenetetramine is used very widely as a therapeutic agent in a number of pathological conditions. A chemical study of this substance was begun with the object of obtaining information with regard to its behavior in the human body. The first problem taken up involved the determination of the amounts which leave the body unchanged under various conditions. The results obtained in this study will be presented in this paper.

After administration, hexamethylenetetramine may be detected in almost all body fluids.<sup>1</sup>

The method of estimating hexamethylenetetramine quantitatively was described in detail in the *Biochemical Bulletin* and will be summarized briefly here.

To the solution in question, 50 cc. being a convenient amount to use, there is added from a burette or pipette with stirring, an alcoholic iodine solution, containing 3.6 g. iodine in 100 cc. 95 per cent ethyl alcohol. No protein or similar substance forming a precipitate with iodine, may be present. If hexamethylenetetramine is absent, no precipitate is formed at first and then on further addition bluish black iodine precipitates. If hexamethylenetetramine is present, a precipitate is formed which is first brownish-yellow in color, and finally reddish to dark brown. About 20 per cent excess iodine is used; if more, iodine is precipitated. The amount needed may be judged by the formation

<sup>1</sup> For references, cf. P. J. Hanzlik and R. J. Collins, *Arch. of Intern. Med.*, 12, 578 (1913).



of the precipitate as the iodine solution is added, or an approximate determination is made first and then a more accurate one with new solution. After all the iodine is added, as a rule 5 to 8 cc. are required, the mixture is allowed to stand ten minutes with occasional stirring and filtered with suction through a Gooch crucible containing an asbestos mat (filtration through paper does not give satisfactory results). Refiltration through the same mat is advisable. The precipitate is washed 5 to 10 times with cold water. The precipitate and crucible may be dried in a vacuum desiccator over calcium chloride to constant weight (no appreciable loss of iodine occurs at room temperature within reasonable time limits) and weighed as tetraiodide. Better than weighing the precipitate is the titration of the iodine present with thiosulphate solution. For this method, the precipitate and crucible are transferred to a beaker, 50 cc. water and 3 cc. glacial acetic acid added, and the mixture titrated with  $\frac{M}{25}$  sodium thiosulphate solution, a few cubic centimeters starch solution being added toward the end. The lumps of the precipitate must be broken up continually with a stirring rod and all of the tetraiodide must be in solution before the end point is considered to have been reached. One cubic centimeter of the thiosulphate solution is equivalent to 0.00507 gm. iodine, or 0.0014 gm. hexamethylenetetramine. If the liquid in which the hexamethylenetetramine is to be determined contains protein matter (such as albuminous urines, etc.), an equal volume of methyl alcohol is added, the mixture allowed to stand one or two hours at room temperature, filtered by decantation through folded filter paper, evaporated to one-third the volume in a current of air or under diminished pressure, iodine solution added and the determination carried out as already described. The presence of glucose does not interfere with the estimation of hexamethylenetetramine.

The results obtained in water solution may be in error 10 per cent of the amount of hexamethylenetetramine present, due to the possibility of the iodine being carried down and of all of the hexamethylenetetramine not being precipitated. In tests with urines, it was found that the error of the determina-

tions was considerably less, not more than 5 per cent as a rule.

If, in testing the urines or other specimens for hexamethylenetetramine with iodine, the result is negative or doubtful, the test is confirmed by acidifying a few cubic centimeters of the solution with sulphuric acid, warming, and then testing with phloroglucin and alkali for formaldehyde. The absence of the latter proves conclusively the absence of hexamethylenetetramine.

Formaldehyde was tested for directly in all the urines examined by adding to  $\frac{1}{2}$  cc. an equal quantity of a 1 per cent solution of phloroglucin in 15 per cent sodium hydroxide, and estimating the concentration of the formaldehyde by the depth of the pink or red color produced.<sup>2</sup> The acidity ( $H^+$  ion concentrations) of the urines was not measured. An indication of this may, however, be obtained from the concentration of the formaldehyde, since it has been shown repeatedly that formaldehyde is not formed from hexamethylenetetramine in neutral or alkaline solution. The presence of formaldehyde therefore indicates a hydrogen concentration greater than  $10^{-7.0}$  its absence one of  $10^{-7.0}$  or less.

The results presented in Table I show the amounts of hexamethylenetetramine excreted by normal (average) persons. The amounts taken (dissolved in 250 cc. water) are indicated in the subdivisions of the tables. Column one gives the name of the subject and number of the experiment. The four parts following consisting of four columns each, give the results of the analyses of the specimens of urine obtained after the ingestion of the hexamethylenetetramine; the first of these columns showing the time after taking the hexamethylenetetramine or after obtaining the last specimen of urine, at which the given specimen was obtained, the next column its volume, the next its specific gravity, and the next, the amount in grams of hexamethylenetetramine found. After these four sets of columns the total amount in grams of hexamethylenetetramine found in the total urine, and the percentage amount recovered are given.

<sup>2</sup> Cf. Hanzlik and Collins, loc. cit.

TABLE I  
The excretion of hexamethylenetetramine in normal urines

NAME	I. SPECIMEN				II. SPECIMEN				III. SPECIMEN				IV. SPECIMEN				FORMALDEHYDE TEST	REMARKS
	After Hrs.	Amt.	Sp. Gr.	Hexam. Pd.	After Hrs.	Amt.	Sp. Gr.	Hexam. Pd.	After Hrs.	Amt.	Sp. Gr.	Hexam. Pd.	After Hrs.	Amt.	Sp. Gr.	Hexam. Pd.		
		cc.		grams				grams				grams					grams	per cent
<i>One gram hexamethylenetetramine taken.</i>																		
A 1	1	380	1.008	0.18	1	387	1.007	0.15	3	435	1.011	0.28	2	112	.023	0.07	0.68	68
2	1	229	11	0.10	1	378	9	0.16	2½	335	13	0.17	2½	380	11	0.08	0.51	51
3	1½	357	9	0.14	2	142	19	0.17	3½	302	19	0.16					0.47	47
4	9	686	16	0.69	4	250	22	0.05	4	365	15	trace					0.74	74
5	9	1080	12	0.58	4½	240	15	0.02	3½	460	14	0.02					0.62	62
6	2	595	12	0.17	2½	446	12	0.25	3	102	24	0.02					0.44	44
7	2½	390	9	0.14	1½	265	13	0.28	2	190	18	0.16	2	232	13	0.09	0.67	67
8	8½	790	12	0.66	3½			trace									0.66	66
B 1	1	110	24	0.04	2	112	23	0.10	2½	90	23	0.07	1½	75	26	0.03	0.24	24
2	1½	84	22	0.03	2½	110	23	0.07	1½	168	14	0.10	2½	160	22	0.05	0.25	25
3	1	224	12	0.05	2½	298	14	0.31	2	308	8	0.17	2	198	18	0.06	0.59	59
4	7½	276	32	0.46	4	174	26	0.05	3½	137	25	0.02					0.53	53
5	2½	144	29	0.01	2½	156	24	0.14	2	130	27	0.03					0.18	18
6	2	193	18	0.10	1½	215	14	0.22	3	175	20	0.06					0.38	38
7	7½	410	24	0.35	4½			trace									0.35	35
C 1	1	210	15	0.11	1½	395	9	0.25	2½	250	20	0.03	1½	170	19	0.04	0.43	43
D 1	1	63	34	0.01	4½	121	32	0.12	1½	47	34	0.01					0.14	14
2	1	112	26	0.03	3½	263	19	0.16	1½	74	23	0.02	1½	184	18	0.09	0.30	30
3	10	287	35	0.17	5½	192	25	0.01	4	100		trace					0.18	18
4	7½	350	27	0.26													0.26	26
E 1	1	480	6	0.05	1½	140	16	0.08	2½	355	8	0.21	2	182	14	0.05	0.39	39
																	Faint	
																	Faint to strong	
																	Fair	
																	Very faint	
																	Very faint	
																	Faint	





The next column shows the results obtained in testing the urines for formaldehyde. In these tests, only those marked "Strong" may be considered to have any bactericidal action, that is to say, to contain formaldehyde in a concentration of at least 1 to 10,000. In the last column, under "Remarks" is shown whether any other substance such as sodium dihydrogen phosphate, sodium benzoate, etc., was taken at the same time as the hexamethylenetetramine.

Although only a limited number of results were obtained (forty in all), certain consistent relationships appear. The lower the specific gravity of the urine, the larger was the amount of hexamethylenetetramine excreted. This is clearly true with the urines of any one individual and best shown by experiments F 1, 2, 3, 4. Here are given the results for four successive days. On alternate days, large quantities of fluid were ingested, giving specific gravities of 1.012–1.013 and excretion of about 50 per cent, on the other days with average amounts of water, the specific gravities were 1.023–1.029 and the excretion 17 per cent to 27 per cent. Similar relations appear in comparing different individuals. With A, the specific gravity was less than 1.020 for all but three specimens, the hexamethylenetetramine excreted was 44 per cent to 74 per cent; for D, specific gravity 1.018–1.035, excretion 14 per cent to 30 per cent; for G, specific gravity above 1.030 as a rule, excretion 12 per cent to 14 per cent; C and E with low specific gravities, about 40 per cent; B, average specific gravity 1.025, 18 per cent to 40 per cent, with one low specific gravity and high excretion (B3). There was one apparent exception to this rule, where a high excretion accompanied a high specific gravity (B4). These results, so far discussed, refer to the tests with one gram hexamethylenetetramine. When smaller amounts were taken, the total excretion was smaller and the regularity was not so apparent. With a greater number of experiments it might appear more clearly. When two grams were taken, the regularity was very apparent again, even with only three experiments.

The amount of formaldehyde found had no apparent relation to the amounts of hexamethylenetetramine taken or excreted.



In the few cases with sodium dihydrogen phosphate and sodium benzoate, no effect was noticed; in the one case with sodium bicarbonate, the urine was alkaline.

The hexamethylenetetramine always appeared in the first hour's urine, but in most of the cases which were studied in this way the amount increased and reached a maximum during the second and third hours. It still continued to appear for some time after this, but practically no more was excreted after 12-14 hours.

Table II shows the results of two series of experiments with continued administration of hexamethylenetetramine. The headings of the columns indicate the significance of the figures. A measure of the relative concentrations of hexamethylenetetramine in the different specimens of urine is given in the last column which shows the volume in cc. of the specimen in question which contains 10 mgm. of hexamethylenetetramine.

With A, 0.67 grams were taken three times a day. Of the total amount (4.00 gms.), 63 per cent was excreted. After the first three hours, the concentration of the hexamethylenetetramine in the urine remained fairly uniform, ranging from 1 part in 1400 to 1 part in 2800. With B, half the quantity was taken, and the total amount excreted 47 per cent. The specific gravity was somewhat higher as a rule than with A. The concentration in the urine varied considerably more than with A, undoubtedly due to the smaller amounts taken.

Since some of the relations between the excretion of hexamethylenetetramine and other properties of the urines of normal (average) individuals are comparatively simple, it seemed of interest to study similarly the urines of patients suffering from kidney and other diseases. The results are summarized in table III. The clinical diagnoses are given in column 2. In columns 3 to 7 are given the data regarding the hexamethylenetetramine excretion. In each case, 1 gram of the substance in 250 cc. water was administered at night before sleep and the urine collected for 12 or 24 hours. The volume of the urine, its specific gravity, the formaldehyde content as shown by the color developed with phloroglucin and alkali, and the total hexamethylenetetramine

TABLE II

*The excretion of hexamethylenetetramine taken for several days*

NAME	DATE	HEXAM. TAKEN	URINE			10 MG. HEXAM. CONTAINED IN CC. URINE
			Vol.	Spec. grav.	Hexam. fd.	
	<i>time</i>	<i>grams</i>	<i>cc.</i>		<i>grams.</i>	
A	March 29					
	9.00 a.m.	0.67				
	11.40		530	1.011	0.09	59
	1.20 p.m.		226	15	0.08	28
	1.25	0.67				
	4.00		261	18	0.15	17
	March 30					
	7.00 p.m.					
	11.00 p.m.		1060	22	0.47	23
	7.00 a.m.					
	9.00	0.67				
	11.15		455	18	0.18	25
	1.45 p.m.		375	15	0.21	18
	2.00	0.67				
	4.30		243	15	0.18	14
	6.30	0.67				
	March 31					
	6.30 p.m. to					
	7.30 a.m.		1640	15	1.14	14
	11.00 a.m.		400	14	0.03	133
	1.30 p.m.		430	9	trace	

Total taken 4.00 grams. Total excreted 2.53 grams. 63 per cent.

B	March 29					
	9 a.m.	0.33				
	10.30 a.m.		100	25	trace	
	1.00 p.m.		285	14	0.13	22
	1.05	0.33				
	1.30		164	4	0.02	82
	3.15		190	14	0.08	24
	5.00, 7.30, 10.50		450	28	0.04	112
	11.00	0.33				
	March 30					
	8.00 a.m.		640	17	0.16	40
	9.30	0.33				
	11.30		302	14	0.07	43
	1.00 p.m.		140	16	0.01	140
	1.05	0.33				
	2.00		250	8	0.08	31
	4.15		192	14	0.09	21
	12.00 p.m.		530	28	0.11	44
	March 31					
	8.00 a.m.		425	16	trace	
	11.30				trace	

Total taken 1.67 grams. Total excreted 0.79 grams; 47 per cent.

found in per cent are given. The eighth column gives the non-protein nitrogen of the blood in milligrams nitrogen per 100 cc. blood. These were determined by Dr. I. Greenwald of this laboratory by the method recently developed by him involving the use of trichloroacetic acid for precipitating the proteins of the blood.<sup>3</sup> The last column gives the results of the tests of the functional activity of the kidneys with phenolsulphonephthalein by the method described by Rowntree and Geraghty.<sup>4</sup> The figures were obtained in the regular course of the examinations in the wards of the hospital.

As stated by Greenwald, the non-protein nitrogen content of the blood normally is not more than 30 mgm. per 100 cc. Any value greater than this indicates a pathological condition. Rowntree and Geraghty found that the ordinary normal limits of the sulphonephthalein test gave an excretion of 60 per cent to 85 per cent of the dye in two hours. Values less than this show an impaired activity of the kidneys. From the results given in Table I it may be concluded tentatively that the excretion of hexamethylenetetramine varies with the specific gravity of the urine within certain limits, and that taking one gram under the conditions described, for normal urines with specific gravities ranging from 1.015 to 1.025 the percentage excreted may be taken to lie between 80 per cent and 40 per cent. With higher specific gravities less would be excreted, with lower more might be excreted.

It is manifestly impossible to draw general conclusions from the limited number of cases which are given in Table III. If the pathological conditions are very striking, the clinical symptoms are readily diagnosed, and the functional tests and other analyses and tests are easily interpreted. This is shown in cases 4, 5 and 8. Here the findings of the dye tests were low, the non-protein nitrogen contents of the blood very large, and practically no hexamethylenetetramine excreted although the specific gravities of the urines were such that normally considerable amounts should have been present. The clinical diagnoses

<sup>3</sup> J. Biol. Chem. 21, 61 (1915).

<sup>4</sup> Jour. of Pharmacology and Experimental Therapeutics, 1, 579 (1910).

TABLE III

*The excretion of hexamethylenetetramine in pathological urines*

NO.	DIAGNOSIS	URINE				N. P. N. MG. PER 100 CC.	DYE TEST
		Vol.	Spec. Grav.	Formal- dehyde	Hexam.		
		cc.			per cent		per cent
1	Atrophic cirrhosis of liver. Nephrotosis. Cholelithiasis. Operation; improved.	430	1.016	Negative	27	33.6	
2	Chronic interstitial nephritis. Improved.	1650	11	Faint	86	38.9	• 44
3	Chronic interstitial nephritis. General arteriosclerosis. Cardiac hypertrophy. Improved.	132 617	13 14	Negative Negative	Trace 6	52.7 67.6	35 35
4	Chronic cardiac insufficiency. Cardiac hypertrophy. Chronic interstitial nephritis. Atrophic cirrhosis of liver. Died.	200	15	Negative	Trace	145.4	32
5	Chronic interstitial nephritis. Chronic uremia. Unimproved.	475 155	14 14	Negative Negative	Negative Negative	164	0
6	Septic infarct of right kidney. Operated after first specimen. Cured. Subacute endocarditis. Improved.	1104 1850 1175 1800 1875 920 483	12 13 12 15 16 14 17	Negative Trace Trace Trace Trace Trace Strong	Trace 88 73 92 100 42 24	49.4	86
7	Chronic interstitial nephritis Improved.	483	17	Strong	24	46.0	59
8	Chronic interstitial nephritis. Chronic uremia, simple anemia. Died.	412	12	Trace	Trace	125 159	10
9	Chronic interstitial nephritis. General arteriosclerosis. Cardiac hypertrophy. Improved.	828	14	Trace	Trace	78.8	26
10	Amyloid kidneys, syphilis. General anasarca. Improved.	360	35	Negative	Negative	30.4	45
11	Chronic interstitial nephritis. Tuberculosis of lungs. Improved.	870	11	Negative	61	24.3	95
12	Chronic endocarditis and nephritis. Improved.	524	14	Negative	9	70.9	45
13	General arteriosclerosis. Chronic gout. Hernia. Improved.	224	12	Faint	9	35.7	97
14	General arteriosclerosis. Cardiac hypertrophy. Detachment of retina. Improved.	318	17	Faint	13		80



TABLE III—Continued

NO.	DIAGNOSIS	URINE				N. P. N. MG. PER 100 CC.	DYE TEST
		Vol.	Spec. Grav.	Formal- dehyde	Hexam.		
		cc.			per cent		per cent
15	Chronic endocarditis (aortitis). Auricular fibrillation. Cirrhosis of liver.	1320	9	Fair	35	36.8	95
16	Chronic myocarditis, with dilatation. Arteriosclerosis. Improved.	746	12	Faint	10		58
17	Leukemia. Died,	240	11	Very faint	12	29.3	93
		292	9	Very faint	19		
18	Suppurative spinal leptomeningitis. Cured.	1800	6	Fair	10	41	94
19	Acute articular rheumatism. Cured.	477	26	Trace	Trace		
20	Tertian malaria. Cured.	700	25	Trace	Trace		
21	Staphylococcus bacteremia without metastases. Improved.	1005	12	Negative	Trace		
22	Pernicious anemia. Improved.	47	20	Faint	1	29.3	
23	Tuberculosis of knee. Improved.	790	26	Negative	Trace	57.9	83
24	Syphilis of base of brain. Improved.	580	14	Strong	25	33.3	45

showed serious kidney conditions and there was no improvement of the patients following treatment. The clinical diagnoses indicated kidney diseases for cases 1-12. Except for 6 and 11, the dye tests confirm these, and except for 10 and 11, and possibly 1, the non-protein nitrogen confirm them as well. With regard to the excretion of hexamethylenetetramine, cases 2 and 11 appear to give the average values. Case 6, for the first determination gave only a trace; after operation, the excretion appeared to be normal. If a conclusion may be drawn from these results, it would appear that certain pathological conditions of the kidneys result in a greatly decreased excretion of hexamethylenetetramine. Case 11 gives normal results by the three methods. It is possible, therefore, that here the clinical diagnosis may have been wrong. Outside of this, case 2 gives results for the dye test and non-protein nitrogen determination showing a pathological condition, while the hexamethylenetetramine excretion was normal. For case 10, the dye and



excretion tests showed impaired functional activity, but the non-protein nitrogen was almost normal. For case 1, the latter was somewhat high, and the excretion somewhat low. Case 7 gives a low excretion, abnormally high non-protein nitrogen, and almost normal dye test.

Cases 13-17 clinically did not present positive evidence of impairment of kidney function, although its possible occurrence was indicated. The dye tests are somewhat high for three of these, for two out of three the non-protein nitrogen determinations are also high, while the hexamethylenetetramine excretions are very low for four out of the five. The last seven cases clinically showed no evidence of kidney involvement. Dye tests and non-protein nitrogen determinations showed impaired renal function and non-protein nitrogen retention in several. In all except the last, the hexamethylenetetramine excretion was abnormally low, while in the last it was well below the average. It is possible that other factors, such as the acidity of the stomach contents, play an important part in determining the amount of hexamethylenetetramine excreted.

No general conclusions can be drawn with regard to the appearance of formaldehyde in these urines. A positive test was obtained in 20 of the 32 examined. The reaction obtained however was strong enough in only two of these (7 and 24) to indicate direct bactericidal action.

One other point was studied in this connection. The possibility of deleterious action due to hexamethylenetetramine or formaldehyde in the urine has been referred to a number of times.<sup>5</sup> No such action was observed in the experiments so far recorded in this paper. A series of experiments was carried out in which hexamethylenetetramine was administered to a dog weighing about eight kilos and the amount excreted in the urine determined. The specific gravity ranged from 1.014 to 1.039 and the formaldehyde test, although distinct as a rule, was in no case strong. With one gram hexamethylenetetramine, the amount excreted varied from 45 per cent to 92 per cent, with

<sup>5</sup> Lately by L. H. Levy and A. Strauss, *Archives of Internal Medicine*, 14, 74 (1914).

two grams from 46 per cent to 92 per cent, and with three grams from 67 per cent to 92 per cent. With three grams per day for four successive days, the urine on the fourth day was found to contain enough blood to produce a red color. On decreasing the amount of hexamethylenetetramine for the next day only a trace of blood was observed, and this disappeared when no more hexamethylenetetramine was administered.

The writers wish to thank Dr. W. W. Herrick, Assistant Physician of Roosevelt Hospital for the interest he has displayed in the work and for his kindness in obtaining the pathological specimens of urine which were examined.

#### SUMMARY

1. A method for the estimation of hexamethylenetetramine is described.

2. The total and percentage amounts of hexamethylenetetramine recovered in the urine of normal (average) individuals after administration by mouth were determined and some of the factors involved in the excretion discussed.

3. In general terms, the amount normally excreted is greater the lower the specific gravity of the urine.

4. The concentration of formaldehyde in urine had no apparent connection with the amount of hexamethylenetetramine excreted.

5. In a number of pathological cases involving impairment of kidney function, abnormally small amounts of hexamethylenetetramine were excreted.



# THE COMPARATIVE PHARMACOLOGIC ACTION OF ETHYLHYDROCUPREIN (OPTOCHIN)<sup>1</sup> AND QUININE

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## INTRODUCTION

Recent European literature contains so many references to optochin from the clinical standpoint and from that of experimental chemotherapy, that it seemed strange that we could find no references to a systematic study of the pharmacologic action of this substance. In view of the extensive publicity that has been given to optochin, especially abroad, it appeared to us desirable to make a pharmacologic study of it, to discover its possible uses, limitations and dangers.

As optochin is chemically related to quinine, we approached our task by undertaking a comparison of this new agent with the old and well-known body.

The chemical name of this substance is ethylhydrocuprein.<sup>2</sup> It is obtained as a substitution product of quinine. Cuprein is an alkaloid contained in small amounts in several cinchona

<sup>1</sup> For convenience, the shorter clinical term "optochin" has been adopted in this paper in preference to the longer chemical name "ethylhydrocuprein."

<sup>2</sup> The specimen we used was obtained from Zimmer & Co. Its melting point is 250° C., as determined by Prof. A. H. Clark of the School of Pharmacy of the University of Illinois.



barks, being somewhat more prominent in *Remijia cuprea* than in others. While quinine is a methoxycinchonin, cuprein is a hydroxycinchonin.

Morgenroth and Halberstaedter (1), making an extensive systematic investigation of the chemotherapeutic action of quinine and of some of its derivatives in experimental trypanosomiasis in mice, found that hydroquinine and ethylhydrocuprein are superior to quinine prophylactically and therapeutically.

Extending their investigations to bacterial diseases, Morgenroth and Levy (2), found that ethylhydrocuprein acts as a specific internal disinfectant against pneumococcus infection in mice, though very nearly fatal doses of the agent had to be used. Morgenroth and Kaufmann (3) found oil solutions of the free alkaloid safer and more efficacious than aqueous solutions of the salt. Gutman (4) and Moore (5) showed that it is equally efficacious against different strains of pneumococci. So specific is the action of this substance on the pneumococci in vitro as compared with other micro-organisms, that Moore (5) suggests it might possibly be used as a test for the pneumococcus.

It can, however, hardly be said that the results were all that could be desired in the hands of all observers. Boehncke (6), for instance, tried to improve the results by combining optochin treatment with serotherapy. He found one strain of pneumococci that seemed to increase the toxicity of optochin to mice. Morgenroth and Kaufmann (3) have shown that pneumococci may be rendered quite immune to optochin by a few passages through mice treated unsuccessfully with the agent.

Wright (7), Tugenreich and Russo (8), and Schiemann and Ischivara (9) have shown that optochin has a highly selective action upon pneumococci in vitro (fatal concentration 1:800,000), even in the presence of serum (fatal concentration 1:400,000). Wright calculated that, in doses of 0.5–1.0 gm., optochin would attain sufficient concentration in the blood stream of the human subject to be fatal to the pneumococcus.

No wonder then that optochin soon came to be used clinically in pneumonia. A number of observers obtained favorable results, among whom may be mentioned: A. Fraenkel (10),

Vetlesen (11) and Kaufmann (12), who found that optochin was of considerable therapeutic value in pneumonia, especially if given early in the case and in guarded doses, e.g., 0.25–0.30 gm., evenly divided during day and night, the total daily dose not exceeding 1.2–1.5 gm. for a few days, because of danger of amaurosis. Lapinski (13) obtained moderately favorable results in early cases. Parkinson (14), Baermann (15), and Waetzold (16), on the other hand, found it of limited value. The last named author summarizing the 135 cases thus far reported finds 43 cases (about 32 per cent) successful, 77 cases (about 57 per cent) doubtful, and 15 cases (about 11 per cent) fatal. He believes that one of its chief effects is an antipyretic action. Waetzold and others agree that there is no doubt that optochin is not capable of destroying pneumococci in the system of the patient; the most that can be hoped from it is that it may exert an unfavorable influence upon the growth of the organisms, if it is administered very early in that disease.

In other pneumococcus infections, optochin has likewise been used with asserted good results. Leschke (17) reports specific results in pneumococcus angina. Introduced into ophthalmology by Goldschmidt (18) in pneumococcus infections of the eye, especially in *ulcus cornea serpens*, it has also been found useful by Schur (19) and Gebb (20). Gradle (21) finds it of use only in superficial corneal ulcers, not in the deep variety. Optochin has been used even in a case of pneumococcus meningitis by intralumbar and intraventricular injection with alleged advantage, though the patient ultimately died (Wolff and Lehmann (22)).

Most recently optochin has also been tried in malaria and found capable of curing cases that had been found resistant to quinine (Izar and Nicosia (23), Liefmann (24)).

It is in view of all these data, that we undertook the pharmacologic study of optochin the results of which we now wish to report.

## 1. TOXICITY OF OPTOCHIN

(a) *On Infusoria*

To compare the effect of optochin with that of quinine upon unicellular organisms, cultures of *Stylonychia*<sup>2</sup> were used. Accurately measured quantities of the infusorial cultures and of the drug solutions were mixed in small glass capsules which were covered to prevent evaporation and observed from time to time.

TABLE I  
*Comparative action of optochin and quinine on Stylonychia*

OPTOCHIN HYDROCHLORIDE		QUININE HYDROCHLORIDE	
Dilution	Result	Dilution	Result
1 : 10,000	+	1 : 10,000	+
1 : 15,000	+	1 : 15,000	+
1 : 18,000	+	1 : 18,000	+
1 : 20,000	+	1 : 20,000	0
1 : 25,000	+	1 : 25,000	0
1 : 30,000	+	1 : 30,000	0
1 : 35,000	+	1 : 35,000	0
1 : 40,000	0	1 : 40,000	0

+ Means all motion stopped within 3 hours. .

0 Means some motion still present after 24 hours.

- ✓ The results given in Table I show that optochin is about twice as toxic as is quinine in its effect on the species studied. Other species that were studied differed from *Stylonychia* in their susceptibility to these agents, but they likewise showed themselves less resistant to optochin than to quinine; the difference was, however, not very great.

(b) *Toxicity for frogs*

Table II shows that the minimal lethal dose of optochin is 0.3 mg. per gm. of frog, while that of quinine is 0.35 mg. per gm.

<sup>2</sup> We are indebted to Prof. A. F. Shull of the Zoölogical Laboratory of the University of Michigan for supplying us with cultures of the various organisms.

TABLE II

*Comparative toxicity of optochin and quinine on Rana pipiens*

OPTOCHIN HYDROCHLORIDE			QUININE HYDROCHLORIDE	
Dose mg. per gm.	No. of experiments	Results	No. of experiments	Results
0.10	1	Recovered	1	Recovered
0.20	3	All recovered	1	Recovered
0.25	5	3 recovered	6	4 recovered
0.30	5	1 recovered	6	3 recovered
0.35	1	Died	5	1 recovered
0.40	1	Died	1	Died

Optochin is therefore somewhat more toxic to the frog than is quinine, the ratio being approximately as 7 to 6.

*(c) Toxicity for white mice*

Again we see from Table III that optochin is more toxic than quinine: the minimal lethal dose of optochin for white mice being 0.5 mg. per gm. while that of quinine is 0.7 mg. per gm., a ratio of 7 to 5.

TABLE III

*Comparative toxicity of optochin and quinine on white mice*

OPTOCHIN HYDROCHLORIDE			QUININE HYDROCHLORIDE	
Dose mg. per gm.	No. of experiments	Results	No. of experiments	Results
0.30	1	Recovered	1	Recovered
0.40	1	Recovered	1	Recovered
0.45	4	Recovered		
0.50	8	6 died	1	Recovered
0.55	4	3 died		
0.60	3	All died	2	All recovered
0.65			3	All recovered
0.70			3	All died

## 2. LOCAL ACTION OF OPTOCHIN ON THE CONJUNCTIVA

A comparative study of the local action of optochin and quinine was made by instillation of solutions of various strengths into the conjunctival sac of rabbits, which showed that optochin is



somewhat more irritant than quinine. This could, however, only be plainly demonstrated by the use of 4 per cent solutions.<sup>4</sup> Such solutions produce violent inflammation, fibrinous exudation and chemosis lasting for 24 to 48 hours: the reaction to quinine being less severe and of shorter duration than that to optochin. 3 per cent, 2 per cent and even 1 per cent solutions of optochin are irritant; 1 per cent solutions of optochin producing a hyperaemia that lasts for several hours.

In addition to the irritation, both substances produce an anesthesia, which has been carefully studied by Morgenroth and Ginsberg (25). These authors found that optochin is  $2\frac{1}{2}$  to 3 times more active as an anesthetic than quinine: an anesthesia lasting half an hour being produced by 1.25 per cent solution of optochin hydrochloride, while it requires a 3 per cent solution of quinine hydrochloride to produce the same result.

### 3. ACTION ON THE NERVOUS SYSTEM

Optochin is a depressant to the upper parts of the central nervous system, apparently affecting the brain first and the medulla next, while the spinal cord is stimulated.

#### (a) *Symptoms in the frog (*Rana pipiens*)*

Soon after the injection, the animal appears depressed; when turned on the back, it is unable to resume its normal position. Its respirations are slowed at first, later occurring at long intervals or when the animal is stimulated. Reflexes are still present, indeed they are frequently at this time slightly exaggerated. Within a few hours, the reflexes are decidedly exaggerated while at the same time a paralysis of the peripheral motor nerve endings becomes more and more evident, so that after a lapse of hours there may be no response or only a single twitch on stimulation of the nerve with the tetanizing current.

That the paralysis is due to involvement of the peripheral motor nerve endings, i.e., a curare action, may be shown by protecting one limb against the action of the poison by a liga-

<sup>4</sup> Made of quinine hydrochloride by the addition of a minimal amount of acid.

ture tied tightly around it with exception of the sciatic nerve, prior to the injection. If 6 or 7 hours after the administration of optochin the sciatic nerves of both limbs are stimulated simultaneously with the same strength of tetanizing current by means of split cord electrodes while the Achilles tendons are attached to weighted writing levers, one writing above the other, a tracing like that shown in figure 1 is obtained.



FIG. 1. MYOGRAM OF FROG'S GASTROCNEMIUS

Poisoned with optochin (lower tracing) compared with that of protected muscle (upper tracing). Stimulation of *sciatic nerve* with tetanizing faradic current.

The protected muscle records a tetanus, while the poisoned muscle gives but a single twitch. That the effect is due to involvement of the motor nerve endings and not of the muscle fiber, is shown by comparison with figure 2.

The unprotected poisoned muscle makes a single twitch while the protected muscle records a prolonged tetanus. If, on the other hand, the muscles themselves are stimulated directly, a prolonged tetanus is obtained from the unprotected as well as from the protected muscle (fig. 2). Evidently the paralysis is not due to a disturbance of the muscle, but to paralysis of the

motor nerve endings. Quinine produces practically identical symptoms. It also paralyzes the motor nerve endings in the later stages of its action (see figs. 3 and 4), a fact which seems to have thus far escaped attention.

(b) *Symptoms in mice*

Optochin like quinine when injected subcutaneously produces a general depression of the animal with convulsions, probably



FIG. 2. MYOGRAM OF FROG'S GASTROCNEMIUS

Poisoned with optochin (lower tracing) compared with that of protected muscle (upper tracing). Stimulation of muscle with tetanizing faradic current.

The protected as well as the unprotected muscle records a prolonged contraction. Evidently the muscle fibers themselves are not paralyzed.

of asphyxial nature, ushering in death due to paralysis of the respiratory center, as the heart can be felt beating after the respiration has ceased.

(c) *Symptoms in rabbits*

The first marked symptom to be noted in this animal from poisoning with optochin is a weakness of the hind legs. This

is followed by weakness of the front legs, sinking of the head and labored respiration, sometimes accompanied by stridor. There is some exaggeration of reflexes. Brief clonic convulsions usually precede death, then respiration stops, the heart continuing to beat for a little while after. Quinine produces practically the same symptoms.

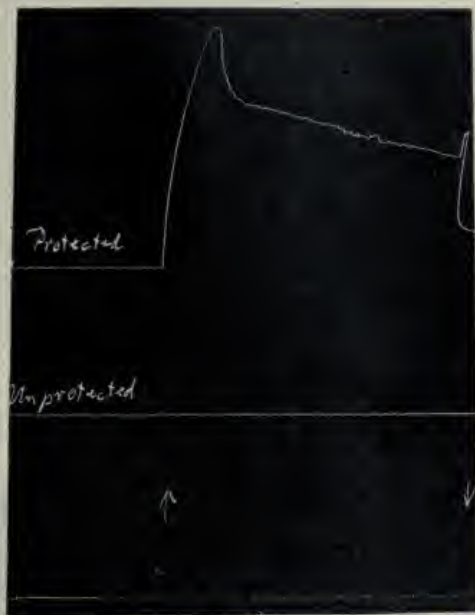


FIG. 3. MYOGRAM OF FROG'S GASTROCNEMIUS

Poisoned with quinine (lower tracing) compared with that of protected muscle (upper tracing). Stimulation of *sciatic nerve* with tetanizing faradic current.

The protected muscle records a tetanus, while the poisoned muscle gives no contraction. That the effect is due to involvement of the motor nerve endings and not of the muscle fiber, is shown by comparison with figure 4.

#### 4. ACTION OF OPTOCHIN ON THE CIRCULATION

In the studies of optochin on the circulation, experiments were made to determine its effect both on the heart and on the vascular system. Its effects on the former were studied by perfusion of the isolated frog's heart and by means of the myo-



cardiogram of the intact dog's heart. The vascular effects were determined by perfusion of frogs and of isolated kidneys of dogs.

From the perfusion experiments of isolated frogs' hearts, the method of which was previously described by one of us (26),



FIG. 4. MYOGRAM OF FROG'S GASTROCNEMIUS

Poisoned with quinine (lower tracing) compared with that of protected muscle (upper tracing). Stimulation of *muscle* with tetanizing faradic current.

The protected as well as the unprotected muscle records a prolonged contraction. Evidently the muscle fibers themselves are not paralyzed.

it appears that optochin has a greater toxic action on the cardiac muscle than quinine. While quinine depresses the cardiac muscle of the frog in the systolic phase, optochin depresses it in both phases, systolic as well as diastolic. (See figs. 5 and 6.)

It is interesting to note in this connection that frogs' hearts

having been perfused with either optochin or quinine do not recover if the drugs are stopped and Ringer's solution is substituted. The explanation of this phenomenon probably lies

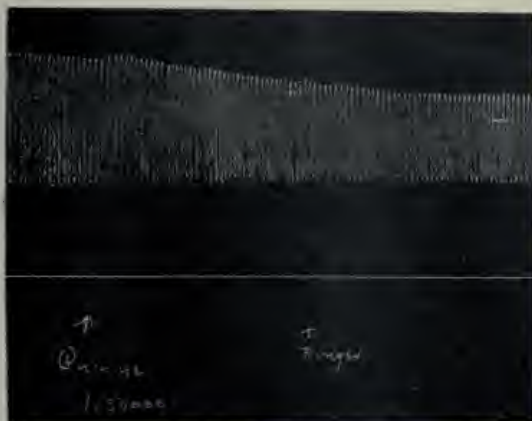


FIG. 5. PERFUSION OF FROG'S HEART. UPSTROKE REPRESENTS SYSTOLE

Shows the effect of quinine hydrochloride 1 : 50,000. Note depression of cardiac muscle in the systolic phase.

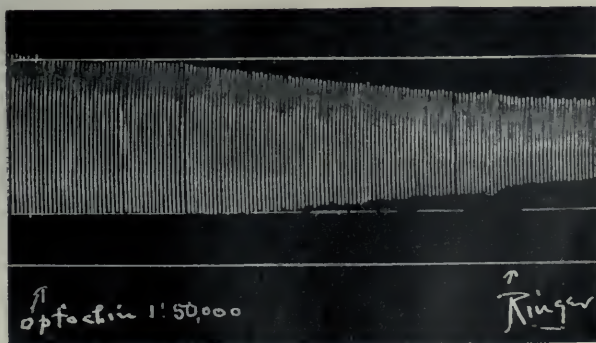


FIG. 6. PERFUSION OF FROG'S HEART. UPSTROKE = SYSTOLE

Shows cardiac depression in both phases caused by a 1 : 50,000 solution of optochin hydrochloride.

in the fact that the drugs named are protoplasmic poisons and so their action on the cardiac muscle cell is of a permanent nature.

The depressant action of optochin on the intact mammalian heart is shown in the accompanying myocardiographic tracing of the dog's heart. Reference to figure 7 will show that 80 mgm. of optochin injected intravenously caused a marked de-

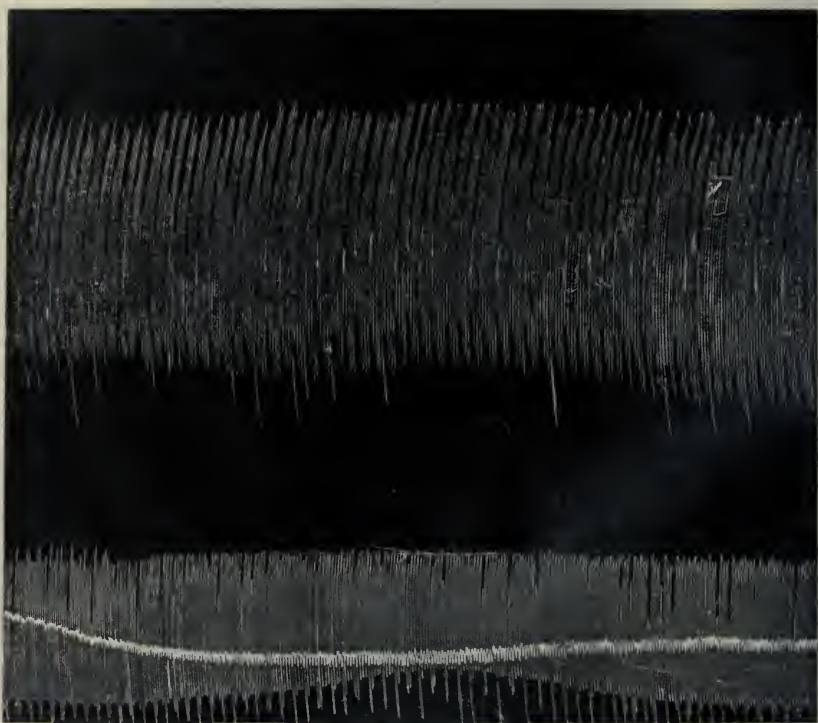


FIG. 7. MYOCARDIOGRAM AND BLOOD PRESSURE TRACING. DOG. UPPER TRACING, AURICLE; LOWER, VENTRICLE

Down stroke = systole. Shows fall in blood pressure and depression of heart, especially the ventricle, caused by the intravenous injection of 80 mgm. of optochin hydrochloride.

pression of the cardiac muscle especially of the ventricle, the auricle showing a tendency toward dilatation. The effect of quinine injected in the same amount intravenously was not nearly so marked.

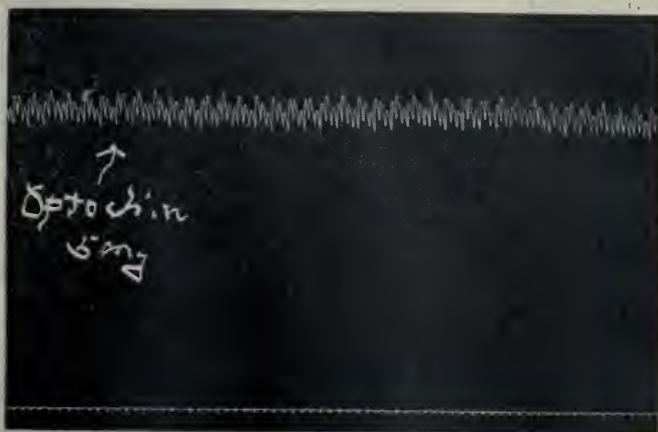


FIG. 8. BLOOD PRESSURE, DOG

Five milligrams of optochin hydrochloride injected intravenously with practically no effect on blood pressure.

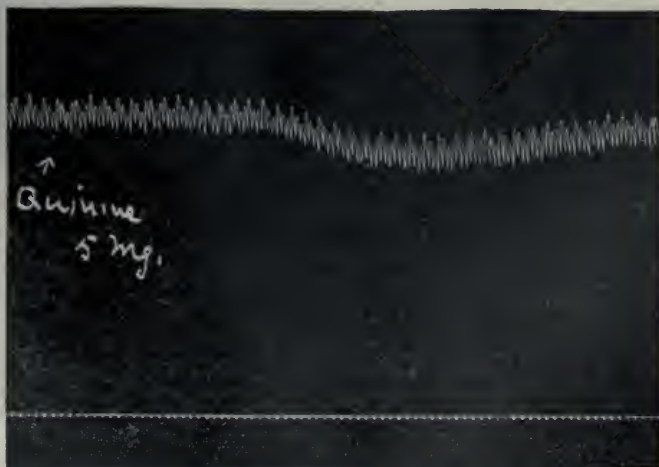


FIG. 9. BLOOD PRESSURE, DOG

Shows a fall in blood pressure from 5 mgm. of quinine hydrochloride injected intravenously.



The fall in blood pressure shown in figure 7 results quite constantly from the intravenous injection of either optochin or quinine, the effect being due in both cases to cardiac depression. Experiments performed to determine the comparative effects of optochin and quinine on the blood pressure of the dog showed that optochin lowers the blood pressure less than quinine. Thus while 5 mgm. of optochin caused practically no change in blood pressure, as is seen from figure 8, 5 mgm. of quinine did lower the blood pressure to about the same extent as 10 mgm. of optochin. (Cf. figs. 9 and 10).



FIG. 10. BLOOD PRESSURE, DOG

Ten milligrams of optochin hydrochloride injected intravenously. Shows some lowering of blood pressure as produced by 5 mgm. of quinine hydrochloride.

These changes in blood pressure occurred alike with intact vagi, cut vagi, as well as the atropinized heart. The lesser effect of optochin on the blood pressure in spite of its greater depressant action on the heart is explained by the peripheral vasoconstrictor effect of optochin, as will be shown presently, so that the fall in blood pressure that would be caused by its cardiac depression is partly counteracted. Thus the condition of the blood pressure cannot be relied upon as a guide to the condition of the heart in optochin administration. Indeed from

some of our observations it would appear that optochin administered intravenously to animals frequently proves fatal even in comparatively small doses, probably owing to this depression of the heart.

To determine the relative action of optochin and quinine on the peripheral vascular system, a number of perfusion experiments were performed. Frogs (*Rana pipiens*) were employed chiefly for these experiments. The brain and the cord were destroyed, the bulbous aorta exposed, and a cannula introduced peripherally, while the ascending vena cava was divided. The animal tied on a frog board was now suitably suspended and physiological salt solution was fed from a reservoir placed at a definite height so as to give a constant hydrostatic pressure throughout the experiment. The outflowing liquid was measured at definite intervals and when a constant rate of outflow was obtained, optochin and quinine solutions of varying strengths were each perfused. A few experiments were also performed on isolated dogs' kidneys, the solutions being fed through a cannula introduced into the renal artery. A typical protocol of one of the experiments will serve to illustrate.

Exp. 7. July 28, 1915. Dog's kidney. Time intervals 3 min.

Physiological salt solution.

1.....	10.0
2.....	11.0; average 10.5 cc.

Quinine hydrochloride 1 : 5000

1.....	11.0 cc.
2.....	11.0
3.....	11.0; average 11.0 cc.

No reduction.

Physiological salt solution.

1.....	13.0 cc.
2.....	13.0; average 13.0 cc.

Optochin hydrochloride 1 : 5000

1.....	7.0 cc.
2.....	9.0
3.....	8.5; average 8.0 cc.

Reduction of 40 per cent.

The results of these experiments are presented for convenience in tabular form. Reference to Table IV will make it evident that while the peripheral vascular action of quinine is very slight, that of optochin is considerable. It is not at all unlikely that the clinical symptom of amaurosis so frequently encountered in the administration of optochin may be due to the peripheral vaso-constriction.

TABLE IV

*Perfusion experiments. Comparative action of optochin and quinine on the vascular system*

OPTOCHIN HYDROCHLORIDE			QUININE HYDROCHLORIDE		
Exp. no.	Concentration	Reduction	Exp. no.	Concentration	Reduction
		<i>per cent</i>			<i>per cent</i>
2A	1 : 5000	63	1A	1 : 10000	12
2B	1 : 5000	58	1B	1 : 5000	5
4	1 : 5000	41	1C	1 : 1000	9
5A	1 : 5000	13	*7A	1 : 5000	0
5B	1 : 5000	6	9A	1 : 500	0
*6A	1 : 5000	40	9B	1 : 250†	20
*6B	1 : 10000	50	10C	1 : 250†	14
*7B	1 : 5000	40			

NOTE: Experiments marked \* were performed on isolated dogs' kidneys, all other experiments were made on frogs.

† It is doubtful whether these results should be considered in comparison with the other figures of the table, for in so high a concentration as 1 : 250, quinine destroys or injures muscle (Secher (27)) which may be responsible for the results. That the outflow continued to diminish instead of increasing when physiological salt solution was turned on, further points to a permanent injury to the blood vessels.

It will not be out of place to state here that two experiments were performed with optochin to see how it compares with quinine in its action on the uterine muscle. The experiments were made on the intact as well as on the isolated uterus of the cat. Though the experiments are too few in number to permit us to draw very precise conclusions, we may say that the results of the experiments we have performed indicate that the action of optochin on the uterus is essentially like that of quinine.

## 5. ACTION OF OPTOCHIN ON TEMPERATURE

A comparative study of optochin and quinine on temperature was carried out on a number of rabbits. Our animals were made

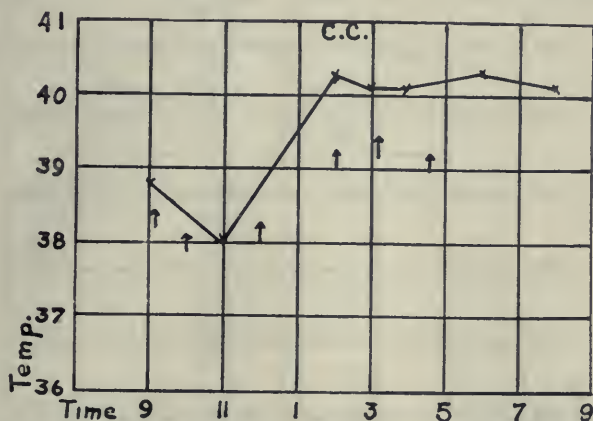


CHART I Fever curve, control. Arrows in this and all the subsequent charts indicate intraperitoneal injections of dog serum to maintain the fever.

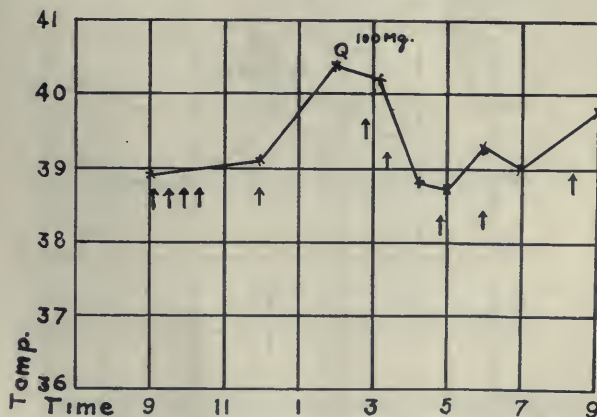


CHART IA Shows the antipyretic effect from 100 mgm. of quinine hydrochloride injected subcutaneously at *q*.

febrile, for the most part, by an anaphylactic procedure, dog serum being used for sensitization.

Briefly stated, we gave our rabbits four to five hourly intra-



venous injections of half to one cc. of dog serum. After an interval of two weeks, we were able to produce and maintain fever in our rabbits by repeated injections of half to one cc. of

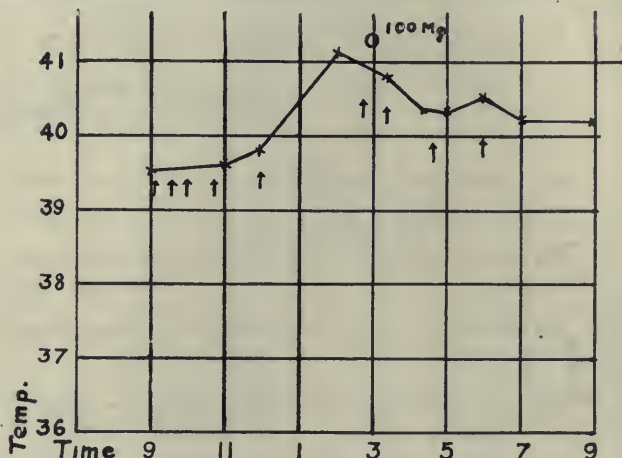


CHART IVA At 100 mgm. of optochin hydrochloride were injected subcutaneously with evidently very little effect.

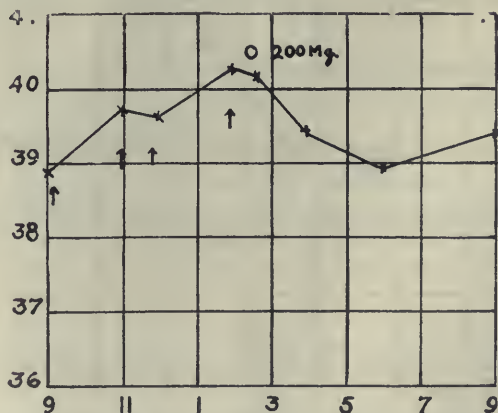


CHART VIIIb Shows the effect of 200 mgm. of optochin hydrochloride, comparable with 100 mgm. of quinine.

dog serum given intraperitoneally. A few of our animals were made febrile by the subcutaneous injection of a single dose of Witte's peptone (about one gram per kg. of body weight).

A few of the fever charts obtained are reproduced here to show that optochin possesses an antipyretic action, although to a less extent than quinine. Thus while quinine produces a good antipyretic effect in 100 mgm. doses, as it appears from Chart IA,

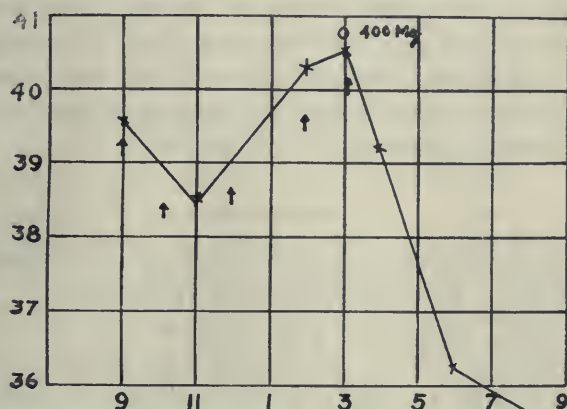


CHART VI At 0 400 mgm. of optochin were injected subcutaneously. This animal developed toxic symptoms and died.

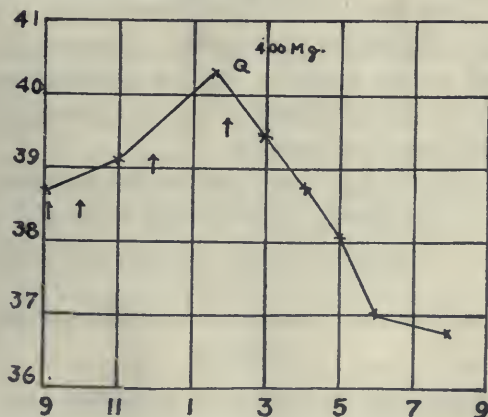


CHART X Shows the effect of 400 mgm. of quinine hydrochloride injected at *q*.

optochin in the same dose produces only a very slight drop in temperature; and it takes twice that dose or 200 mgm. of optochin to produce a definite reduction in temperature (see Charts IV A and VIII B). On the other hand, when a large dose of

optochin is given, such as 400 mgm., a condition of collapse with prolonged subnormal temperature is obtained frequently with a fatal outcome, as is shown in Chart VI. (Cf. this with Chart X). Chart I is presented as a control.

Summing up our observations on the antipyretic action of optochin, we should say that in small doses it is uncertain in its action, in moderate doses it is less efficient than quinine, and that its effective antipyretic dose may give rise to collapse, because it closely approaches its toxic dose.

#### CONCLUSIONS

1. The pharmacologic action of optochin is qualitatively like that of quinine. The differences are quantitative.

2. Optochin is more toxic than quinine. In view of its considerable degree of toxicity, we would urge caution in the clinical use of optochin, especially in its intravenous administration.

3. Optochin administered intravenously lowers the blood pressure somewhat less than quinine, though it depresses the heart more. The peripheral vaso-constriction probably counteracts partly the effects of cardiac depression. The blood pressure is not a safe guide in the administration of optochin.

4. Optochin is less efficient as an antipyretic than quinine in experimental fever of rabbits.

5. Lastly, since it has been advocated for local use in eye infections, it is important to note that even a 2 per cent solution of optochin is highly irritant to the conjunctiva.

#### *Note on a fatal case of pneumococcic endocarditis treated with optochin*

Since this paper has been written, a clinical case treated with optochin in one of the large hospitals has come to the notice of one of us. This patient, a male 21 years of age, had pneumococcic endocarditis and ran a septic temperature. Optochin was administered intravenously in doses of 0.1 gm., 0.2 gm. and 0.3 gm. within an interval of 10 hours with apparently no effect. On the following day at 9 a.m. 0.5 gm. was given and

at 1.30 another dose of 0.5 gm. was given, whereupon the patient complained of numbness of lower extremities, impaired vision and inability to hear. (Unfortunately no fundus examination was made at that time.) His radial pulse was feeble. A blood culture taken at this time subsequently showed a reduction from 100 to 4 colonies per cc. The patient's temperature however was not appreciably affected by the treatment. At 8.30 of the following day 0.4 gm. of optochin was again given and at 11.20 0.2 gm. Patient soon became unconscious and died within one hour of the last administration.

There seems little room for doubt that, while optochin proved here very efficacious in reducing the number of pneumococci in the blood, it may have contributed to the fatal result. Death was undoubtedly due to failure of the circulation, to which the cardiac depression produced by the drug probably contributed, for the symptoms prior to the fatal ending point to optochin intoxication and weakened circulation.

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## DOES THE PITUITARY GLAND CONTAIN EPINEPHRIN OR A COMPOUND SIMILAR TO IT?<sup>1</sup>

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• The pressor action of extracts of the pituitary gland was first called attention to by Oliver and Schaefer.<sup>2</sup> Later Howell claimed that the pressor action was confined to the posterior lobe, and that extracts of the anterior lobe (sheep) produced little or no effect on the blood pressure (dog.)<sup>3</sup> This was corroborated by Schaefer and Vincent.<sup>4</sup> On this basis, preparations of the posterior (infundibular) lobe are said to be marketed, but as the process of separating the anterior and posterior lobes is tedious and expensive, it is doubtful "whether in all cases, preparations said to be made from the posterior portion are really not prepared from the gland as a whole."<sup>5</sup>

The work of Biedl and of Lewis, Miller, and Matthews tends to show that the pressor compound of the pituitary gland is found in the pars intermedia and that in separating the anterior and posterior lobes, portions of the pars intermedia are removed with them, so that extracts of the anterior or of the posterior lobe, or of the pars intermedia may produce a rise in blood pressure.<sup>6</sup>

<sup>1</sup> This investigation was supported by a grant from the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.

<sup>2</sup> Oliver, G. and Schaefer, E. A.: *Journ. Physiol.*, vol. 18, p. 277, 1895.

<sup>3</sup> Howell, W. H.: *Journ. Exper. Med.*, vol. 3, p. 245, 1898.

<sup>4</sup> Schaefer, E. A. and Vincent, S.: *Journ. Physiol.*, vol. 25, p. 87, 1899.

<sup>5</sup> Heidelberg, F., Pittenger, P. S., and Vandekleed, C. E.: *Journ. Amer. Pharm. Assoc.*, vol. 3, p. 809, 1914.

<sup>6</sup> Lewis, D., Miller, J. L. and Matthews, S. A.: *Arch. Int. Med.*, vol. 7, p. 785, 1911; Biedl, A. *Innere Sekretion.*, vol. 2, p. 114.

In Lewis, Miller, and Matthew's experiments,  $33\frac{1}{3}$  per cent of the dogs, injected with extracts of the anterior lobe taken from the pituitary gland of cattle, responded by a rise in blood pressure, while a rise occurred in 55 per cent of those injected with extracts of the posterior, but Herring<sup>7</sup> who carefully separated the anterior lobe of such pituitaries, claims that extracts of them exert no pressor action on the cat and have only slight action on the virgin-rat uterus, while extracts made from the pars intermedia contract the rat uterus, but do not raise the blood-pressure in cats. However Herring believes that the active principle "is almost certainly a product of the cells of the pars intermedia, but is passed into the pars nervosa, either as a secretion from the cells, or by the actual conversion of the epithelial cells into hyaline bodies and granular débris" (p. 262).

As epinephrin is the most active pressor compound known to occur in the animal organism, it would be logical to infer, either that pituitary extracts owe their blood-pressure raising effect to an action on the suprarenal glands, whereby an increased amount of epinephrin is discharged into the circulation, or that a compound similar to epinephrin or that epinephrin itself occurs, at least to some extent, in the pituitary gland.

Against the first view it may be argued that occlusion of the suprarenal circulation does not diminish the pressor effect of the injections of pituitary extracts.<sup>8</sup>

The following observations have been urged against the view that the pituitary gland contains epinephrin:

1. All the color reactions for epinephrin which have been tried on pituitary extracts have proved negative.<sup>9</sup>

2. Aqueous extracts of the posterior lobe of the pituitary gland, unlike epinephrin, slow the heart rate, and some claim that slowing occurs even after atropinization. The rise in blood pressure is more persistent than that from epinephrin.

<sup>7</sup> Herring, P. T.: *Quart. Journ. Exper. Physiol.*, 1915, vol. 8, p. 245.

<sup>8</sup> Hoskins, R. G. and McPeck, C.: *Amer. Journ. Physiol.*, vol. 32, p. 241, 1913.

<sup>9</sup> Houssay, B. A.: *Estudios sobre la acción de los extractos hipofisarios*, p. 159; Allers, R.: *Muench. Med. Woch.*, vol. 56, pt. 2, p. 1474, 1909.

3. They constrict strips of the coronary vessels, which epinephrin does not constrict.<sup>10</sup> This has also been found to occur in the coronary vessels of the isolated rabbit heart perfused by the Langendorff method as modified by Locke.<sup>11</sup>

4. A tolerance to the pressor action of pituitary extract has been reported (Howell). Tolerance has not been reported for pure epinephrin.

5. Unlike epinephrin, extracts of the hypophysis cause contraction of both the pregnant and non-pregnant cat's uterus and even where hypogastric nerve stimulation causes relaxation.<sup>12</sup>

6. Hypophyseal extracts are claimed not to cause vasomotor reversal after ergotoxin injection (Dale).

7. These extracts cause contraction of isolated loops of intestines (dog).

8. Physiological experiments would indicate that a decoction of the posterior lobe of the pituitary gland owes its pressor effect mainly to an action on smooth muscle fibers of the blood-vessels, and not on the "receptor bodies" of the sympathetic vaso-constrictor nerves, as in the case of epinephrin; and according to Dale the view of the identity of the pressor action of the pituitary gland with that of epinephrin is "wholly illusory and superficial." According to Dale "there is an increasing body of evidence which indicates that the pituitary principle acts on plain muscle more by increasing its sensitiveness to normal stimuli than by acting as a direct stimulant."

These arguments indicate the absence of epinephrin in pituitary extracts, provided such extracts represent a single pure compound, but it has been shown that extraction of the *dried* glands with alcohol removes one depressor compound,<sup>13</sup> and there is some reason to believe that pituitary extracts may contain a second depressor compound which is not removed by alcohol, hence in pituitary extracts there must be one or two

<sup>10</sup> De Bonis, V. and Susanna, V.: *Zent. f. Physiol.*, vol. 23, p. 169, 1902.

<sup>11</sup> Dale, p. 430.

<sup>12</sup> Dale, p. 436, see also Lieb, C. C.: *Amer. Journ. Obstetrics*, vol. 71, 1915.

<sup>13</sup> Lewis, Miller and Matthews: l. c.; Shaefer and Vincent: *Journ. Physiol.*, 1899, vol. 25, p. 87.



depressor compounds, and one or more pressor compounds along with colloids, etc.

Herring claims that extracts of the pars intermedia contract the virgin rat's uterus, yet fail to raise the blood-pressure in cats, suggesting the presence of several active compounds in the pituitary gland. This view is also supported by the fact that extracts of elasmobranch (skate) pituitary, which contains no posterior lobe, exert a galactagogue action, yet do not raise the blood-pressure.

According to Herring, extracts of the pars nervosa "produce an immediate and prolonged expansion of the kidney; which, after a longer latent period, is accompanied by an increase in the amount of urine secreted," whereas extracts of the pars anterior, or of the pars intermedia, do not produce this action, so that the principle which acts on the kidney is different from that which acts on the uterus and mammary gland, hence the action of extracts of the pituitary gland would depend upon the predominance of one or other of these substances; in fact in our earlier work aqueous extracts of pituitary gland usually produced a fall, and this was the experience of Silvestrini.<sup>14</sup>

Some time ago we became convinced that epinephrin, or a compound similar to epinephrin, was responsible for much of the pressor action of the pituitary gland, and that this action was probably modified by the presence of one or more active compounds along with colloids, etc.

For isolating epinephrin from suprarenal glands, Weidlein's method offers several advantages.<sup>15</sup> This method slightly modified was tried on the pituitary glands as follows: 1 kilogram of frozen, *untrimmed* beef pituitaries<sup>16</sup> was ground and extracted with a mixture of 1 liter of 95 per cent commercial ethyl alcohol, 100 cc. of chloroform, and 50 cc. of glacial acetic acid. The filtrate, after concentration in vacuo at 55° C. to about 60 cc., was precipitated with four or five times its volume of 95 per cent

<sup>14</sup> See Biedl, vol. 2, p. 134.

<sup>15</sup> Weidlein, E. R.: Journ. Indust. and Eng. Chem., vol. 4, p. 636, 1912.

<sup>16</sup> The pituitary glands were obtained through the courtesy of Miller and Lux, Inc., of San Francisco.

ethyl alcohol. After evaporation to about 60 cc. the fluid was put aside in a closed bottle, with chloretone as a preservative; after 4 or 5 days, when all precipitation had ceased, the filtrate was neutralized with NaOH and concentrated to 60 cc. (Solution A). In some cases HCl was used in modifying the Weidlein method, i.e., in place of 50 cc. glacial acetic acid, 1 cc. of concentrated HCl being used, and the HCl being removed by  $\text{PbCO}_3$ , and the excess Pb by  $\text{H}_2\text{S}$ . This was neutralized by NaOH just before using (Solution B). Extracts corresponding in mode of preparation and strength to the commercial preparation pituitrin were also used. (Solution C.)<sup>17</sup>

Solutions A and B, when only slightly colored, give a greenish blue reaction with iron chloride much like that given by some suprarenal extracts. When the solution is colored the iron chloride gives a blue or black color. Vulpian in describing the reaction between iron chloride and suprarenal extract says, "*Le sesquichlorure de fer et les sels de sesqui-oxyde de fer y produisent une teinte glauque, quelquefois noiâtre, tirant un peu sur le bleu on sur le vert.*"<sup>18</sup> To produce the green reaction it was necessary to add 0.4 cc. (1-1000 adrenalin) to 0.5 cc. of Solution A or B. Below this concentration iron chloride gave a blue or black color. Several samples of commercial pituitrin gave a distinct green reaction with iron chloride.

A dilute acetic-acid extract of the whole fresh pituitary glands was coagulated with heat and precipitated by lead subacetate. If this filtrate was acidulated with acetic acid,  $\text{H}_2\text{S}$  could be used to remove the excess of lead, and the filtrate retained much of its pressor action, but if  $\text{H}_2\text{S}$  was passed into the alkaline solution, the pressor compound could not be detected by blood-pressure methods. The acid filtrate after  $\text{H}_2\text{S}$ , when colorless gave, like suprarenal extract, a pink color with iodine, by Comessatti's reaction, also with potassium persulphate.<sup>19</sup> Gold chloride is reduced by pituitary extract (Solution A or B) as by ex-

<sup>17</sup> See Crawford, A. C. and Ostenberg, Z.: Amer. Journ. Pharm., vol. 86, p. 300, 1914.

<sup>18</sup> Vulpian: Comp. Rend. Acad. des Sci., vol. 43, p. 663, 1856.

<sup>19</sup> Ewins, A. J.: Journ. Physiol., vol. 40, p. 323, 1910.

tracts containing epinephrin, sometimes giving a blue, sometimes a pink or green color. Folin and Denis' uric-acid reagent and diazobenzene sulphonic acid (Pauli's reaction) act with these solutions as with a weak suprarenal solution.<sup>20</sup> Phosphomolybdic acid acts with Solutions A and B as with suprarenal extracts producing a green color and a blue precipitate.<sup>21</sup> Other chemical reactions of epinephrin as with  $\text{AgNO}_3$ , Fehling's solution,  $\text{MnO}_2$  etc. are given by Solutions A and B. In the case of solution C, gold chloride, Folin and Denis' reagent and Pauli's reagent give merely a weak reaction. The other reactions are not constant. This is probably a question of dilution.

The shades of color given in these tests must be influenced by admixed substances, hence to compare the reactions one must use suprarenal extracts from which epinephrin has been largely removed by  $\text{Na}_2\text{CO}_3$ . In such solution the color reactions more closely resemble those given by pituitary extract than with pure epinephrin.

As in the case of epinephrin, pituitary extract seems to lose its pressor action on oxidation. Pituitary extracts, which give the above mentioned color reactions, after oxidation with  $\text{MnO}_2$ , fail to give them, and then only give a weak gold chloride reaction and a weak reaction with Folin and Denis' uric acid reagent.

A solution corresponding to pituitrin was oxidized for 1 hour with  $\text{MnO}_2$  at room temperature. This caused no rise in systemic blood-pressure, but increased to some extent the renal volume (dog). The same extract unoxidized caused a marked rise in blood-pressure and marked increase in the renal volume.

Using Folin and Denis' reagent, it was found that 1 kilo of untrimmed fresh pituitary glands (beef) assayed for 36 mg. of epinephrin, assuming epinephrin was present in the pituitary glands.<sup>22</sup> This amount is too small to be thrown out by alkalis as in the method used for isolating epinephrin from extracts of the suprarenal glands. Using Aldrich's method with 5 kilos of untrimmed frozen beef pituitaries, no precipitate correspond-

<sup>20</sup> See also Aldrich, T. B.: Journ. Amer. Chem. Soc., vol. 37, p. 203, 1915.

<sup>21</sup> Moore, B.: Journ. Physiol., vol. 17, p. xvi, 1895.

<sup>22</sup> Folin, Cannon and Denis.: Journ. Biol. Chem., vol. 13, 477, 1912-13.



ing to epinephrin was obtained. Negative results were obtained with 5 kilos by Abel's method.<sup>23</sup> Ten kilos of untrimmed glands were extracted overnight with a mixture of 5 liters of absolute alcohol, 500 cc.  $\text{CHCl}_3$ , 250 cc. glacial acetic acid. The filtrate was coagulated by heat and then concentrated in vacuo at  $50^\circ \text{C.}$  to about 40 cc. and precipitated with five times its volume of commercial ethyl alcohol (95 per cent). This precipitation was performed three times, and on final concentration (20 cc.) the assay corresponded to 36.5 mg. epinephrin, suggesting a loss during purification. This solution gave no active precipitate with ammonia, even on the addition of 40 mg. adrenalin<sup>24</sup> to this solution.

Under these conditions the proof for the presence of epinephrin in pituitary glands must at present rest on color reactions and on physiological tests. Ewins "found that aminoethanol-catechol (arterenol), as well as dihydroxy-phenylethylamine and its N-alkyl derivatives (including epinine) give the various reactions with about the same degree of sensitiveness as adrenalin."<sup>25</sup> The pressor action of arterenol corresponds to that of natural epinephrin.<sup>26</sup>

We have found that di-hydroxyphenylethylmethylamine (epinine) differs from epinephrin in that when oxidized with  $\text{MnO}_2$ , the solution turns pink, then red, and finally green. Epinephrin does not yield the green color.

Slowing of the heart rate cannot be considered a proof of the absence of epinephrin in pituitary extracts, as slowing may be due to the depressor compound and this might mask the accelerator influence. In Schaefer and Vincent's experiments, the cardiac slowing was not constant, and after atropinization very little slowing occurred, while Einis reported that the first effect

<sup>23</sup> Abel, J. J.: Amer. Journ. Pharm., vol. 75, p. 311, 1903.

<sup>24</sup> The term Adrenalin has been used instead of epinephrin whenever the commercial preparation was used.

<sup>25</sup> Barger, G.: Simpler natural bases, p. 91; Ewins, A. J.: Journ. Physiol., vol. 11, p. 317, 1910.

<sup>26</sup> Schultz, W. H.: Adrenalin and adrenalin-like bodies. Hygienic Bull., no. 55, 1909.



of pituitary extract upon the heart was a diminution in frequency, but that this was followed by an increase.<sup>27</sup>

According to Lewis, Miller, and Matthews, "the pressor effect caused by the injection of extracts of the purest form of the pars intermedia—the fringe adhering to the anterior lobe after separation of the two lobes of the gland—is not associated with slowing of the pulse," and "it seems not improbable that the pars nervosa contains a specific substance which produces it," i.e., effect on the pulse rate. BurrIDGE claims that "adrenalin has a twofold action on the perfused heart of the frog, a primary depressing action, and a secondary favoring action," and that the action depends on the amount of calcium in the perfused fluid.<sup>28</sup>

Active solutions of pituitary glands, if carefully oxidized by passage of air, lose their pressor action, and such solutions then cause lowering of the blood-pressure and slowing of the heart rate (dog), and if an epinephrin solution is added in amounts sufficient to cause a rise about equal to that produced by the original pituitary extract, the curve will be similar to that produced by pituitary extract.

It is usually claimed that pituitary extracts contract rings of the coronary vessels, while epinephrin relaxes them, and this is believed to be strong evidence that the pressor compound of the pituitary is different from epinephrin. However Cow<sup>29</sup> states that in his experiments with pituitary extracts on rings of "cerebral and pulmonary arteries, no appreciable effect was produced; with the coronary artery an indefinite result was obtained, sometimes constriction and sometimes dilatation." The coronary vessels are believed to receive dilator fibers from sympathetic nerves, while the vaso-constrictors are derived from autonomic nerves (vagi).<sup>30</sup> In the light of Fröhlich and Pick's experiments on the intestines, the difference in results of experiments on the coronary vessels are intelligible as pituitary extracts contain a

<sup>27</sup> Einis, W.: *Biochem. Zeits.*, vol. 52, p. 96, 1913.

<sup>28</sup> BurrIDGE, W.: *Journ. Physiol.*, vol. 48, p. xxxix, 1914.

<sup>29</sup> Cow, D.: *Journ. Physiol.*, vol. 42, p. 135, 1911.

<sup>30</sup> *Mass. Arch. f. ges. Physiol.*, vol. 74, p. 281, 1899.

compound which stimulates autonomic and a compound which stimulates sympathetic nerve terminals; hence the results would vary according to the predominance of either factor.

We have noted tolerance to the pressor action in only one preparation, i.e., a preparation made by the modified Weidlein's method. This solution gave tolerance in both a cat and a dog. In the cat there was practically no change in the blood-pressure after the first injection, but in the dog, after one injection, the subsequent injections were always followed by marked lowering of blood-pressure.<sup>31</sup> These experiments suggest that the depressor principle, or principles, in the pituitary extract may sensitize to the depressor action. The tolerance may really be a neutralization of the pressor effect by the depressor.

In reference to the pressor tolerance resulting from the injection of pituitary extracts, Howell says: "The loss of reaction following upon repeated injections seems to be much more marked than in the case of adrenal extracts" (p. 253). On the isolated uterus, pituitary extracts produce no tolerance.<sup>32</sup> It may be remembered that the injection of d-adrenalin is said to produce a condition in which l-adrenalin causes no pressor action. However d-adrenalin has not yet been found in nature, but has only been obtained by synthetic means. A tolerance to the glycosuric action of epinephrin seems to occur, thus the injection of epinephrin into dogs or man causes glycosuria, but "the effect is always reduced by repeating the injection."<sup>33</sup>

Longitudinal strips of the non-pregnant cat's uterus, suspended in 200 cc. of oxygenated Locke's solution at 39° C., were treated with 3 cc. of Solution A. This produced marked contraction. After about 12 minutes the addition of 1 mg. adrenalin caused relaxation. The strip was then washed and fresh Locke's solution used. On adding a mixture of 1.5 cc. of Solution A with 1 mg. adrenalin the strip contracted, although not so much as with pituitary extract alone. After rewashing the strip and changing the Locke's solution, 1 mg. adrenalin now caused sim-

<sup>31</sup> See also Vincent, S.: *Internal Secretion and the Ductless Glands*, p. 378.

<sup>32</sup> Fröhlich, A.: *Wien. Med. Woch.*, 1914, p. 1061.

<sup>33</sup> Von Noorden, C.: *Practitioner*, vol. 94, p. 234, 1915.

ple relaxation. After pituitrin, the uterus (rabbit) responds more to hypogastric nerve stimulation than before its use (v. Frankl-Hochwart and Fröhlich).

We found in decapitated cats, that Solution A or C and pituitrin (tried once), produced vaso-motor reversal after ergotoxin, like epinephrin. If Dale's theory of vaso-motor reversal is correct, we would argue that our pituitary extract acted on sympathetic nerve terminals.<sup>34</sup>



FIG. 1. Decapitated cat (weight, 1 kilogram) injected intravenously with 40 mg. ergotoxin (commercial). 1, time in seconds; 2, signal; 3, base line; a, injection of 1 ampoule pituitrin (Parke, Davis & Co.); b, injection of 2 cc. NaCl solution. This pituitrin had previously caused a rise in blood pressure.

Of the biological tests for epinephrin, that with strips of the small intestine is considered the most distinctive and perhaps the most sensitive.<sup>35</sup> On isolated longitudinal strips of cat or rabbit intestine, solution A first increased the muscle tone, then lowered it (rabbit). If epinephrin was mixed with this solution

<sup>34</sup> See also Fröhlich, A., and Pick, E. P.: Arch. f. exper. Path., vol. 74, p. 114, 1913.

<sup>35</sup> Hoskins, R. G.: Journ. Pharmacol., vol. 3, p. 95, 1911.



the character of the reaction was practically unchanged. A control solution of pure epinephrin caused merely relaxation to normal of contracted strips (cat). Pituitary extracts, when oxidized by  $\text{MnO}_2$  at room temperature, lose their pressor action, yet still contract longitudinal strips of cat's or rabbit's intestine and also longitudinal strips of the cat's uterus.

In Bayer and Peter's experiments with circular strips of the small intestines (rabbit) suspended in oxygenated Ringer's solution, pituitrin, after a few seconds, caused a diminution of rhythmic contractions. This was associated with diminution in tone, but not so marked as in the case of adrenalin.<sup>36</sup> Using small amounts of pituitrin (0.5 cc. in 15 cc. Ringer's solution) the diminution might last for a minute and be followed by increase of contraction with increase of tone. Larger amounts of pituitrin (4 or 5 cc. in 15 cc. Ringer's solutions) which cause loss of rhythm did not cause the secondary enlargement of the peristaltic wave, but the increase in tone occurred.

The initial inhibition is traced by Bayer and Peter to a stimulation of the sympathetic terminals. The second action they believe due to an action on autonomic nerve terminals, which are less sensitive to pituitrin than are the sympathetic. The increase in tone is due to an action on the autonomic apparatus, because this action is greatly lessened by the previous use of atropin.

As pituitrin does not relax these strips after a maximal adrenalin action, while adrenalin still relaxes after a maximal pituitrin relaxation, Bayer and Peter argue that pituitrin acts on the sympathetic terminals central to where adrenalin acts, but this is not proved. So far as their experiments go they indicate that the inhibitory action is due to the action of the portion of the gland insoluble in alcohol, while the portion soluble in alcohol increases the contractions.

Emphasis has been laid on the view that the action of pituitary extract is on smooth muscle, not on the nerve terminals supplying them. But the experiments of Cow in which he

<sup>36</sup> Bayer, G. and Peter, L.: *Archiv. f. exper. Path.*, vol. 64, p. 204, 1911.



found that rings of the renal artery distal to its origin responded less to pituitary extracts than those taken from the artery closer to its origin, and by Pal's experiments<sup>37</sup> in which the distal rings relaxed, might argue against a direct muscular action. Our vaso-motor reversal experiments and the work of Bayer and Peter point to an action on nerves, rather than on muscle fibers.

Like epinephrin, pituitary extracts cause mydriasis in some animals, and in some animals glycosuria.

It is sometimes stated that pituitary extracts do not cause arterial degeneration, as does epinephrin, but Dale states that Harvey has obtained arterial degeneration from them.<sup>38</sup>

Fröhlich and Pick point out that the action of pituitary extracts on the rabbit respiration consists of an epinephrin-like action on the medulla and a stimulant action on the pulmonary vagus terminals.<sup>39</sup> v. Frankl-Hochwart and Fröhlich claim that on organs on which both pituitrin and adrenalin act, pituitrin acts like a weak adrenalin solution.<sup>40</sup>

A sample of Solution A was assayed with Folin and Denis' reagent, and it was found that each cubic centimeter reacted for 0.675 mg. of epinephrin. A portion was diluted one-half with distilled water. The assay then reacted for 0.337 mg. in each cubic centimeter. 0.337 mg. adrenalin was added to 1 cc. and the assay showed the epinephrin content to be 0.675 + mg. Solution A, and also its dilution to which adrenalin had been added, were tested on decapitated cats by the blood-pressure method, and the curves were found to be similar (prolonged rise), save that the pressor action of the second was slightly more marked, perhaps due to the fact that by diluting the first the depressor action was lessened.

Arterenol is the only known compound which has a pressor activity about that of epinephrin, and which might have been substituted for epinephrin, but in solution this rapidly loses its

<sup>37</sup> Pal: Wien. Med. Woch., 1909.

<sup>38</sup> Dale, p. 431.

<sup>39</sup> Fröhlich, A., and Pick, E. P.: Arch. f. exper. Path., vol. 74, p. 92, 1913.

<sup>40</sup> v. Frankl-Hochwart, L., and Fröhlich, A.: Arch. f. exper. Path., vol. 63, p. 355, 1910.

activity<sup>41</sup> and according to Barger and Dale<sup>42</sup> this compound still exerts a pressor action after ergotoxin, i.e., does not cause vaso-motor reversal.

About a year ago Fühner claimed that various pressor compounds could be precipitated from pituitary extracts by means of phosphotungstic acid, however phosphotungstic acid is not a specific precipitant, but precipitates compounds of high molecular weight such as peptones, also amines and even ammonia.<sup>43</sup> The precipitate, obtained by phosphotungstic acid (Fühner's method) from suprarenal extracts exerts a pressor action, and gives the iron chloride reaction.

#### SUMMARY

1. Pituitary extracts prepared by certain methods give color reactions similar to those given by suprarenal extracts.

2. Slowing of heart rate (dog) was noted from a preparation in which no pressor action was detected.

3. Pituitary extracts cause a strong contraction of non-pregnant cat's uterus. A certain amount of epinephrin when mixed with pituitary extract, does not change the characteristic action of pituitary extract on the cat's uterus.

4. After ergotoxin injection, pituitary extract causes a lowering of blood-pressure as by epinephrin. This suggests that some of the action of pituitary extract is on the sympathetic nerve terminals.

5. Isolated loops of rabbit's and cat's intestine are strongly contracted by pituitary extract. Relaxation of the intestine was not obtained by the addition of a certain quantity of epinephrin to such extracts.

6. By oxidation with  $\text{MnO}_2$ , pituitary extracts lose their pressor action and some of their color reactions, but retain at least to

<sup>41</sup> Schultz, W. H.: Adrenalin and adrenalin-like bases. Hygienic Lab. Bull. No. 55, 1909.

<sup>42</sup> Barger, G. and Dale, H. H.: Journ. Physiol., vol. 41, p. 49, 1910.

<sup>43</sup> Guggenheim, M.: Biochem. Zeits., vol. 65, p. 193, 1914.

some extent their action for both non-pregnant cat's uterus and rabbit's and cat's intestine.

7. Several methods for isolation of epinephrin were tried on the pituitary glands, but were unsuccessful.

#### CONCLUSIONS

Pituitary extracts, when prepared by certain methods, yield color reactions which would suggest the presence of epinephrin or an epinephrin-like compound, and the physiological actions of such solutions can be explained by the presence of such a compound, but modified by admixed substances. Epinephrin has not yet been isolated from these glands, and this may be due to the small amounts present, which would not be precipitated by the method now used for obtaining epinephrin.

# ON THE VASO-CONSTRICTIVE ACTION OF SERUM ON THE CORONARY VESSELS OF THE MAMMALIAN HEART

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Cushny and Gunn (1) recently showed that when the isolated heart of a rabbit has been perfused with Ringer's solution, the addition of serum or plasma to the Ringer reduces the flow through the coronary vessels, and in consequence of this marked changes follow in the cardiac contractions. These changes occur even when the animal's own serum is employed. They ascribed this action not to any new substances having been formed in the serum, but to the vessels having been altered in their reaction through the previous perfusion of the Ringer's solution. Others have supposed that new vaso-constrictor substances, not present in the blood, are formed in serum and cause the constriction of vessels through which they are perfused (Handovsky and Pick (2) ).

I have attempted to determine how far the effects observed by Cushny and Gunn may be obtained with other proteins and colloids and also to determine which constituents of the serum are responsible for the vaso-constriction in the coronary arteries.

I have perfused the hearts of cats and rabbits by the same method as was described by Cushny and Gunn. The heart was first perfused with Ringer's solution for 15-20 minutes, and then the solution to be examined was substituted at the same pressure and temperature; the fluid was oxygenated by a current of oxygen throughout the experiment.

The results of my experiments are shown in the tables given below, in which the rate of flow is indicated by the number of



seconds elapsing between each discharge of Condon's tipper, which held 1.6 cc. The second line gives the rate of the heart per minute in each of these intervals, and the third line the height of the excursion of the ventricle lever.

The first series of experiments was performed with horse-serum in order to familiarise myself with the method. These merely confirmed the observations of Cushny and Gunn and need not be entered on further. The cat's heart reacted in the same way as the rabbit's, both to horse-serum and to cat's serum.

I can also confirm the observation that egg white has the same vaso-constrictor and cardiac action as serum.

*Soluble starch and dextrin.* In these experiments the soluble starch (amylodextrin) was dissolved in distilled water by heating,

TABLE 1

	RINGER'S			5% DEXTRIN IN RINGER'S								RINGER'S					
Rate of flow in secs.	16	17	17	17	20	16	17	16	17	16	16	19	16	15	14	14	16
Heart rate.....	108	108	108	108	108	108	108	108	106	106	106	96	96	90	90	90	90
Heart height.....	7	7	7	7	8	10	11	12	13	14	14	13	13	12	12	10	9

and then Ringer's salts were added. The commercial dextrin (achrodextrin), which contained some quantity of grape sugar, was purified by boiling with alcohol twice, dissolving in a little distilled water, and then precipitating by alcohol again. This was repeated twice, and the purified dextrin was then dried at a low temperature. This dextrin was easily dissolved in Ringer's solution, producing a viscous, colourless, or brownish solution of neutral reaction. The results of one of my experiments with 5 per cent dextrin perfused through a rabbit's heart are shown in table 1.

Both solutions had a considerable viscosity, but they had no distinct effect upon the heart. The coronary flow was never diminished by their perfusion. From these facts it may be seen how little influence increased viscosity in the perfused solution has upon the heart and its vessels. Cushny and Gunn noted some constriction of the coronary arteries in one experiment in

which starch was perfused, but this may have been from mechanical obstruction by the particles of starch.

*Glutin.* Gelatin was extracted with very dilute potassium hydrate solution (0.1 per cent) for 24 hours and the swollen mass was washed successively with dilute acetic acid, water and alcohol. This gluten was then dissolved in warm water, filtered, and precipitated by alcohol. After being dried, it was dissolved to 1 per cent in Ringer's solution by moderate heating and its action on the heart was then examined; it was found to have no effect upon the heart and its vessels. It is unnecessary to give the details of this negative experiment, which resembled those of the starch and dextrin perfusions.

*Gum arabic.* A 4 per cent solution of gum arabic was dialysed in distilled water till the dialysate was free from salts, and was then made up with Ringer's salts and used to perfuse a cat's heart which had previously been perfused with pure Ringer's solution. It failed to cause any vaso-constriction, and in fact rather accelerated the flow through the coronaries.

*Peptone Witte.* Peptone Witte was dissolved in distilled water by heating and then made up with Ringer's salts; the result was as follows.

TABLE 2

1 per cent peptone Witte. Rabbit's heart.

	RINGER'S		PEPTONE IN RINGER'S							RINGER'S						
Rate of flow in secs...	85	85	86	72	40	36	45	45	43	54	54	50	50	50	54	72
Heart rate.....	133	133	139	108	75	68	85	85	84	102	95	108	108	96	102	136
Heart height.....	17	17	18	22	30	35	35	31	28	25	22	20	20	20	19	18

The result of this experiment agrees with that of Handovsky and Pick, who described a distinct vaso-dilator action of the peptone on the frog's vessels by Trendelenberg's method.

According to Popielski (3) the accelerating action of Witte's peptone on the heart does not depend upon the peptone itself, but upon the ash contained in this preparation and especially upon the calcium content. I therefore dialysed the 1 per cent slightly alkaline solution of the peptone in distilled water against

distilled water for eight days, till the dialysate was completely free from salts. Ringer's salts were then added to the fluid in the dialyser and its action on the heart and its vessels was observed. The result was as follows.

TABLE 3  
*1 per cent Witte's peptone, dialysed, in Ringer. Rabbit's heart*

	RING- ER'S		PEPTONE—RINGER'S							RINGER'S							
Rate of flow in secs. ....	54	55	54	45	32	28	26	24	25	28	31	42	48	42	45		
Heart rate .....	100	100	105	100	105	105	108	105	100	100	100	100	100	100	100		
Heart height .....	33	33	33	33	33	28	24	22	22	23	26	28	33	36	38		

Thus the completely salt-free peptone solution also dilates the coronary vessels, so that its action must be ascribed to the direct effect of the non-dialysing constituents of this complex substance.

*Agar-agar* in saturated solution was dialysed in flowing distilled water for seven days till free from chloride, and then was made up with Ringer's salts and perfused through the rabbit's heart. The coronary flow was accelerated by about 50 per cent as soon as the agar-agar solution reached the heart and the rate and strength of the ventricle were augmented. Agar-agar therefore dilates the vessels like peptone.

These experiments show that the vaso-constrictor action on the coronaries is confined to the sera and eggwhite, while various other colloids are either inactive (soluble starch, dextrin, gluten, gum arabic), or even cause coronary dilation (peptone, agar-agar). It is obvious therefore that the slight increase in the viscosity does not explain the phenomenon.

#### PHYSICAL AND CHEMICAL PROPERTIES OF THE VASO-CONSTRICTOR SUBSTANCE IN THE SERUM

*1. Stability against heating.* Horse serum 10 per cent in Ringer's solution was heated in a boiling water-bath for half an hour and when cool was filtered off. In another experiment, in order to provide against a possible precipitating action of Ringer's salts on the vaso-constrictor substance, 10 cc. of the

serum was added to 90 cc. of water, heated, cooled and filtered. The liquid was then brought up to the original quantity, made up with Ringer's salts, and used to perfuse a rabbit's heart with the following results (Table 4).

TABLE 4

	RINGER'S			HEATED SERUM IN RINGER'S			RINGER'S					
Rate of flow in secs.....	66	66	66	70	154	190	154	91	53	59	68	
Heart rate.....	77	77	77	77	70	64	60	60	73	77	81	
Heart height.....	26	26	27	28	29	22	20	20	23	26	26	

The other method of preparation described gave similar results. The vaso-constrictor substance in the serum therefore retains its action after being heated to nearly the boiling point for half an hour, although much of the protein was precipitated; this is contrary to the results obtained by Handovsky and Pick. The precipitation was of course not complete.

2. *Shaking with absorbents.* Boymond (4) observed that the shaking of a solution containing proteins with some indifferent substances causes a precipitation of proteins, and Handovsky (5) found that blood serum shaken with Kieselgur, Kaolin, etc., had a lower viscosity than unshaken serum.

I shook the serum with Kieselgur or Kaolin for three to eight hours and then compared the action of the filtrate on the heart and its vessels with that of the unshaken solution and of a solution which was shaken for the same time without any absorbent. All three solutions caused vaso-constriction and the shaken sera appeared more powerful than the untreated.

But these comparative experiments are of little value for, as Cushny and Gunn pointed out, a heart which has been perfused for a long time with Ringer's solution generally responds to the action of serum more strongly than a fresh heart, and one that has been perfused once with serum previously often shows distinctly greater susceptibility to subsequent perfusion with it. But the action on the coronary flow was not diminished by shaking the serum, though this diminished the viscosity considerably.



3. *Fractionation and separation of the proteins in the serum.*

(1) 50 cc. of normal horse serum was dialysed in parchment paper against distilled water for 5 days, until the dialysate was completely free from chloride; toluol or chloroform was added to prevent putrefaction. The whole dialysate was reduced in vacuo at 37°C. to 50 cc. and diluted with Ringer's solution up to 250 cc., which thus contained 20 per cent of the salts, etc., of the original serum (A). The liquid remaining in the parchment-together with the precipitated globulin was divided into two parts, each of 25 cc. One of these was immediately diluted with Ringer's solution up to 125 cc. (20 per cent) (B). The other was filtered off and the insoluble globulin was washed with distilled water and then dissolved in 125 cc. of Ringer's solution (C). The last filtrate was diluted with Ringer's solution to 125 cc. (D) (the albumin fraction). All the solutions soon became clear after adding Ringer's solution, and then their action on a rabbit's heart was tested with the results indicated in table 5.

TABLE 5

A (dialysate).....	52	52	48	45	43	47	54	60	54	52		
B (remainder).....	46	43	43	45	74	78	76	70	56	60	53	49
C (globulin).....	37	39	40	47	55	48	41	40	40	46	46	43
D (albumin).....	66	64	62	204	208	138	67	67	72	70	68	66

In this table the rate of flow during the perfusion with the various serum preparations is given in italics, that under Ringer's solution in ordinary type.

These results show that the active substance in the serum dialyses with great slowness if at all, and that the solution containing serum albumin retains the vaso-constrictor action upon the heart, while the redissolved globulin fraction has only an insignificant effect upon it.

(2) The globulin and albumin were salted out from the serum by Hammarsten's method and their action and that of the remaining solution was tested on the heart.

50 cc. of normal horse serum was saturated with powdered magnesium sulphate at 30°C. The precipitate was washed with saturated solution of  $MgSO_4$  and was then dissolved in 500 cc.

of distilled water (serum globulin, corresponding to 10 per cent serum). The filtrate and the washings were saturated with ammonium sulphate and the precipitate was washed with saturated solution of ammonium sulphate and dissolved in 500 cc. of distilled water (serum albumin corresponding to 10 per cent serum). The last filtrate, which showed neither the precipitation reactions of proteins nor the biuret reaction, was brought up to 500 cc. Each of these three solutions was liberated from the salts by dialysis in flowing distilled water for 8 days. Ringer's salts were then added and the action of each on the cat's heart was examined. The results of this are given in table 6, which is arranged in the same way as table 5.

TABLE 6  
(Rate of flow)

Globulin....	14	15	15	17	17	15	15	13	12	12	10	10	10	10	10	12	14
Albumin.....	39	39	42	44	39	35	31	26	25	25	25	25	26	29	32	32	
Protein-free solution.....	14	15	16	18	17	17	15	15	15	15	14	16	18	14	14	16	

Here neither the solutions of albumin or globulin nor the filtrate from them had any vaso-constrictor action but, on the contrary, the proteins tended to slightly dilate the vessels. This can only be explained by the proteins having lost their specific property by having been precipitated by salts.

I obtained the same results by the treatment of serum with ammonium sulphate by Pohl's method. The serum solution, from which the globulin was removed, retained its effect upon the heart vessels apparently in undiminished strength, whilst the liquid from which both globulin and albumin had been removed had scarcely any effect.

Finally I separated the proteins from serum by Hardy and Gardiner's method (6) to find whether the proteins or the protein-free filtrate possess the specific action on the heart and its vessels. 50 cc. of normal horse serum, cooled to 0°C., was added to 200 cc. of alcohol previously cooled to - 8°C. After some hours the precipitate was filtered off at 0°C., and washed with cold anhydrous ether until the alcohol was removed completely; it was then thoroughly extracted with boiling ether

several times and dried over  $\text{H}_2\text{SO}_4$  in vacuo. The product, a white powder, was dissolved in 500 cc. of Ringer's solution (corresponding to 10 per cent dilution of the original serum), centrifuged and used for an experiment. The whole filtrate, containing alcohol and ether, was evaporated in vacuo until the alcohol and ether had disappeared, and was then dissolved in 50 cc. of distilled water which was added to 450 cc. of Ringer's solution and centrifuged.

The protein solution proved to have very little constrictor action on the coronary vessels of the heart, though these responded readily to untreated serum both before and afterwards. The experiment was repeated several times with similar results. The serum proteins prepared by precipitation by alcohol-ether by Hardy's method had thus lost in large part their specific action on the heart, though there remained a slight residue of action.

The filtrate from the proteins was also tested on the heart and found to have a varying effect, sometimes weakly constrictor, generally quite negative; this may perhaps arise from the filtrate being very slightly alkaline when measured by Sørensen's method ( $\text{PH}' = 8.04$ ).

In another experiment acetone-ether was used instead of alcohol-ether (Hardy and Gardiner) and the protein was found to be readily soluble in water and Ringer's solution. I dissolved this protein in Ringer's solution in a strength corresponding to 20 per cent of the original serum, and tested its action on the heart vessels. In this experiment, the action of the pure protein was very distinct, whilst the filtrate had no vaso-constrictor but rather a vaso-dilator effect. My failure to obtain the constrictor action from the proteins, prepared by alcohol-ether, was thus due to the difficulty of the manipulation in all probability. Hardy and Gardiner found that the antigen characteristics of the proteins were preserved by their method and I can now add that the action on the coronary vessels also remains intact in their precipitated proteins.

The action of serum on the heart vessels is thus to be attributed to the colloids, while the dialysate of serum does not con-



strict the coronary vessels. The albumin fraction of serum possesses the constrictor action, but it is not certain whether the globulin shares in it or not, for it was impossible to test the globulin fraction alone except after it had been precipitated with salts, and salt precipitation destroys the activity of the albumin and may equally affect the globulin. Proteins denaturated by precipitation with salts or alcohol lost their action on the cardiac vessels, while those prepared by Hardy's acetone-ether precipitation method remained toxic. Heating up to 99° for thirty minutes did not remove the activity of the proteins that remained in solution.

Eggwhite was examined in the same way as serum and gave very similar results when heated, shaken with absorbents, or fractionated by dialysis.

### III. MECHANISM OF THE ACTION OF SERUM ON THE HEART

I have shown above that the action on the vessels is not due to the slight increase in viscosity, for several other colloidal solutions of high viscosity have no such action, and the serum, which has been shaken with absorbents and which has a considerably lower viscosity, acts also with at least the same strength as the original serum.

The action seems inseparable from the proteins, for when serum is dialysed either against distilled water or Ringer's solution, the dialysate fails to contract the coronary vessels or may even dilate them. It may be remarked here that the constriction of the coronary vessels by proteins is of interest in relation to the tests for adrenalin in the blood. A good deal of difficulty has been met in distinguishing the action of serum as such from that of adrenalin by perfusion through the frog's limbs, or the mammalian organs, but there can be no such difficulty in the case of the heart, for here serum causes vaso-constriction, while adrenalin almost invariably causes pronounced vaso-dilatation. I have observed these opposite effects of serum and adrenalin in the rabbit's heart in several experiments.

It is well known that an acid dilates, and an alkali constricts the vessels. The coronary vessels also obey this general rule.



For instance, one drop of dilute acetic acid or of sodium hydrate solution in 100 cc. of Ringer's solution brings about a well-marked change in the flow of the coronary vessels and in the action of the heart. The changes which are caused by an alkali are quite similar to those produced by perfusion with serum in Ringer's solution.

It therefore occurred to me that the action of serum on the heart might arise from the serum possessing a slightly more alkaline reaction than the Ringer's solution, or perhaps from the proteins playing the rôle of a buffer and thus mitigating the action of the H ions. To settle this question, I determined the alkalinity of the Ringer's solution in use by Sørensen's (7) method. I then added 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 cc. of  $\frac{N}{20}$  sodium hydrate solution each to 100 cc. of the Ringer's solution and determined the absolute alkalinity and the hydrogen ion concentration of each mixture; its effect upon the coronary vessels and the heart was then tested. To estimate the hydrogen ion concentration by Sørensen's method the phosphate mixture was used as standard solution; indicators: paranitrophenol, rosolic acid, *p*-benzol-sulphonazo- $\alpha$ -naphthol,  $\alpha$ -naphtholphthalein, neutral red, and phenolphthalein.

Ringer's solution	PH' 7.3
No. 1. Ringer's solution 100 cc. + 0.1 cc. $\frac{N}{20}$ NaOH; PH' 7.7	
No. 2. Ringer's solution 100 cc. + 0.2 cc. $\frac{N}{20}$ NaOH; PH' 8.1	
No. 3. Ringer's solution 100 cc. + 0.3 cc. $\frac{N}{20}$ NaOH; PH' 8.3	
No. 4. Ringer's solution 100 cc. + 0.4 cc. $\frac{N}{20}$ NaOH; PH' 8.5	
No. 5. Ringer's solution 100 cc. + 0.5 cc. $\frac{N}{20}$ NaOH; PH' 8.6	
No. 6. Ringer's solution 100 cc. + 0.6 cc. $\frac{N}{20}$ NaOH; PH' 9.1	

The limit of the vaso-constrictor action of these Ringer's solutions containing NaOH varied somewhat in different hearts. But, generally speaking, solutions Nos. 1 and 2 had no effect upon the coronary vessels, while No. 3 and No. 4 sometimes produced constriction and sometimes failed of effect; Nos. 5 and 6 always induced well-marked changes, which were quite similar to those produced by serum perfusion in the contraction of the heart and the flow through the coronary vessels.

If the action of serum arises from a change in the hydrogen ion concentration therefore, this must amount to about 8.5-9. But there is a general consensus of opinion that the hydrogen ion concentration of serum is very little greater than that of distilled water, its  $\text{PH}'$  amounting on the average to 7.2-7.7 at  $18^{\circ}\text{C}$ .

The normal horse serum employed in my experiments, determined by the electro-chemical method (Michaelis and Davidoff (8) ), had a value of  $\text{PH}' = 7.21$ , while Ringer's solution had a value of  $\text{PH}' = 7.33$  at  $18^{\circ}\text{C}$ ., and the addition of the serum to Ringer's solution made only a very slight change in the  $\text{PH}'$  value and never increased its alkalinity. It is well known that the hydrogen ion of blood is not sensitive to the addition of bicarbonate, on which the alkalinity of Ringer's solution depends. For example, if bicarbonate is added to blood in the proportion of 0.01 mol. per litre, the hydrogen ion concentration suffers practically no change at all. These observations appear to negative the suggestion that the serum action on the heart and coronary vessels arises from a change in the alkalinity.

#### SUMMARY

1. Of the colloids examined, only serum and egg-white proved to possess the characteristic vaso-constrictor action on the coronary vessels, with the accompanying changes in the heart beat, while soluble starch, dextrin, gluten, arabin, agar-agar and peptone, either had no effect or in some cases dilated the coronary vessels.

2. The colloids of serum and egg-white are the constituents which possess this action, and these lose their efficiency when they have been precipitated by salts or alcohol; but the proteins which are not precipitated by boiling temperature (in feebly alkaline media) continue to act, and Hardy and Gardiner's pure proteins also preserve their action when prepared by acetone-ether.

3. The effect of these proteins on the flow is very similar to that of dilute alkali; but when alkali is used, the change in the hydrogen ion concentration which is necessary to cause this

action is far greater than that observed when serum is added to Ringer's solution. The action of serum on the coronary circulation and heart therefore cannot be explained by the serum changing the alkalinity of the perfusing fluid.

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# QUININE AND ATOPHAN IN INFLAMMATION OF FROG'S MESENTERY

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## INTRODUCTION

It is a well-known fact that quinine inhibits the inflammatory process and the migration of leucocytes in the mesentery of frogs. This was pointed out firstly by Binz (1) and his collaborators and subsequently by other authors. Martin (2) confirmed these observations not only on frog's mesentery but also on frog's liver. He injected 2.5 mgm. quinine subcutaneously and repeated the dose after 6 hours. He found this dose effective though he seems not to have seen an entire inhibition, but only remarkable diminution of the migration of leucocytes.

Lately Starkenstein and Wiechowski (3) showed on the conjunctiva of the rabbit's eye that atophan has also an antiphlogistic action.

The following experiments were carried out in order to ascertain the effect, if any, of combining these two drugs, having a view to the possibility of a synergistic action.

## METHOD

The method was that used by Cohnheim (4) in his original experiments. The drug was injected subcutaneously into the thigh of a pithed frog and the animal was left for half an hour, so that the injected fluid might be thoroughly absorbed. Then the abdomen was opened and the mesentery spread on a microscope slide and arranged so that the tissues could be observed continually under the microscope. The hydrochloride of quinine



was used in solutions of different dilution according to the dose to be injected. A tablet of atophan (Schering) containing 0.5 gram was emulsified with 10 cc. of water and the required dose obtained by further dilution when necessary.

In the normal mesentery thus exposed to the air the first migration of leucocytes begins in about  $\frac{1}{2}$  to  $1\frac{1}{2}$  hours (Cohnheim, l. c.).

To be certain of the presence of synergism between quinine and atophan it was necessary to know first the effective and the ineffective dose of each of them. I therefore carried out a series of experiments with each drug alone before I started to experiment with a combination.

#### I. EXPERIMENTS WITH QUININE ALONE

I tried doses from 0.62 mgm. up to 4.37 mgm. per frog. In a dose of 1.25 mgm. or smaller quinine had no effect on the migration of leucocytes, the exudation set in quickly and severally just as in normal frogs (Table I, frogs Nos. 12, 13, 14, 15, 16, 17, 18). When the dose was raised a little to 1.56–1.87 mgm., the inhibitory effect of quinine was evident, the amount of migration was remarkably small and its first setting in much postponed. Further with this dose the circulation was only slightly affected (frogs Nos. 9, 10, 11). Increasing the dose to 2.0–2.5 mgm. did not entirely stop the exudation, there was some migration but only minimal. On the other hand the circulation was severely affected with this dose (frogs Nos. 3, 4, 5, 6, 8, see also Appert (5) and Pekelharing (6)). To ascertain if any dose of quinine stops the migration entirely and at the same time to avoid the effect of larger doses upon the circulation, I tried giving the drug in successive doses (frogs Nos. 1, 2, 7), but the results were the same, the quinine did not entirely inhibit the migration, whilst on the other hand it severely affected the circulation. It appears then that quinine has no effect on the migration in doses of 1.25 mgm. or less, its effective dose begins at about 1.56 mgm. for a frog of about 15 grams; beyond this up to 2.5 mgm. could be given without stopping the circulation,

TABLE 1  
(Experiments with quinine alone)

NO.	WEIGHT OF FROG	DOSE	DEGREE OF EXUDATION	MIGRATION OF WHITE CELLS FIRST SEEN AFTER	OBSERVATION TIME	REMARKS
	gram	mgm.		hours	hours	
1	16.5	I 1.8 II 2.5 Total 4.3	Moderate	1	4½	Circulation defective. Interval between injections 1 hour. Before second injection already minimal exudation.
2	16.5	I 1.87 II 0.63 Total 2.50	Minimal	4½	5¼	Circulation little affected. Interval between injections 2½ hours. Before second injection no exudation.
3	13.7	2.5	Minimal	3¼	4½	Circulation defective.
4	14.0	2.5			1	Circulation defective, it stopped entirely after one hour.
5	10.7	2.5			1	Circulation defective, it stopped entirely after one hour.
6	Not weighed	2.5	Entire inhibition		7½	Circulation quite normal.
7	13.5	I 1.63 II 0.62 Total 2.25	Minimal	3	4	Circulation little affected. Interval between injections, 2½ hours. Before second injection some signs of exudation.
8	11.5	2.0	Moderate	1¼	2	Circulation defective.
9	14.2	1.87	Minimal	4	4	} Circulation little affected.
10	13.0	1.62	Moderate	3	4	
11	11.8	1.56	Moderate	3	4	
12	12.5	1.25	Severe	1¼	5	} Circulation quite normal.
13	9.7	1.00	Severe	1	3	
14	13.3	0.75	Severe	½	1½	
15	Not weighed	0.70	Severe	1	4	
16	Not weighed	0.70	Severe	1½	5	
17	13.0	0.62	Severe	1	1	
18	13.5	0.62	Severe	1	1	

though even the largest doses do not entirely inhibit migration. The smallest effective dose of quinine alone is about 1.56 mgm. per frog of 15 grams. These results are put together in Table I.

## II. EXPERIMENTS WITH ATOPHAN ALONE

75 mgm. of atophan pro frog inhibited the migration entirely without affecting the circulation. 12.5 mgm. proved itself equally effective. 6.25 mgm. had no effect at all on the inflammation. The minimal effective dose of atophan appears then to be between 6.25 and 12.5 mgm. (Table II).

TABLE II  
(Experiments with atophan alone)

NO.	WEIGHT OF FROG	DOSE	DEGREE OF E. A. UDATION	MIGRATION OF WHITE CELLS FIRST SEEN AFTER	OBSERVATION TIME	REMARKS
	<i>gram</i>	<i>mgm.</i>		<i>hours</i>	<i>hours</i>	
1	16.5	95.0	Inhibition		4	Circulation normal.
2	20.0	75.0	Inhibition		7	Circulation at first quite normal, after 3 hours a little affected.
3	18.5	12.5	Some signs	$\frac{1}{2}$	$\frac{1}{2}$	After $\frac{1}{2}$ hour the second dose 37.5 mgm. given and it stopped the migration.
4	14.3	12.5	Inhibition		4	Circulation little affected.
5	15.0	12.5	Inhibition		4	Circulation normal.
6	12.8	6.25	Severe	$1\frac{1}{2}$	2	Circulation normal.
7	12.5	6.25	Severe	2	$2\frac{1}{2}$	Circulation normal.

## III. EXPERIMENTS WITH THE COMBINATION OF QUININE AND ATOPHAN

The drugs were injected separately but into the same limb and with as small a time interval as possible. For these experiments I took as the dose of atophan 2.08 mgm., i.e.,  $\frac{1}{3}$  of its ineffective dose, this dose was used in all experiments and only

that of quinine was varied (Table III). At first  $\frac{1}{8}$  of the ineffective dose of quinine (0.16 mgm.) was combined with atophan.

TABLE III  
(Experiments with the combination of quinine and atophan)

NO.	WEIGHT OF FROG	DOSE	DEGREE OF EXUDATION	MI. RATION OF WHITE CELLS FIRST SEEN AFTER	OBSERVATION TIME	REMARKS
	gram	mgm.		100,000	100,000	
1	14.8	Quinine 0.5 Atophan 2.08	Inhibition		4	Circulation quite normal.
2	12.5	Quinine 0.43 Atophan 2.08	Inhibition		4 $\frac{1}{2}$	
3	13.8	Quinine 0.43 Atophan 2.08	Inhibition		3 $\frac{1}{2}$	
4	11.3	Quinine 0.37 Atophan 2.08	Moderate	$\frac{1}{2}$	1 $\frac{1}{2}$	Circulation interfered with by accidental pressure on mesenteric veins.
5	12.0	Quinine 0.31 Atophan 2.08	Inhibition		3	Circulation quite normal.
6	12.5	Quinine 0.31 Atophan 2.08	Minimal	2 $\frac{3}{4}$	4	
7	12.5	Quinine 0.31 Atophan 2.08	Moderate	3 $\frac{1}{2}$	4	
8	11.0	Quinine 0.16 Atophan 2.08	Minimal	4	4	Circulation only slightly affected.
9	13.2	Quinine 0.16 Atophan 2.08	Severe	$\frac{1}{4}$	$\frac{1}{2}$	Circulation quite normal.
10	12.5	Quinine 0.16 Atophan 2.08	Inhibition		3 $\frac{1}{2}$	
11	12.3	Quinine 0.16 Atophan 2.08	Severe			Mesentery found inflamed on exposure, increased quickly afterwards.



The results were not conclusive as of four frogs two showed more or less inhibition and two none at all (frogs Nos. 8, 9, 10, 11). Afterwards  $\frac{1}{4}$  of the ineffective dose of quinine (0.32 mgm.) was combined in the same way with atophan and this mixture gave almost entire inhibition or at least considerable postponement (frogs Nos. 5, 6, 7). Finally the dose of quinine was increased to  $\frac{1}{3}$  of its ineffective dose (0.4–0.5 mgm.) and this in combination with atophan caused entire inhibition (frogs Nos. 1, 2, 3), except frog No. 4, which showed moderate inflammation, but there was an accidental interference with circulation, probably affecting absorption. This combination does not affect the heart at all. It is quite evident that quite small doses of quinine and atophan ( $\frac{1}{3}$  of an ineffective dose) when combined can manifest a very strong action in inhibiting the migration of leucocytes, stronger even than that of any dose of quinine alone which could be given.

#### SUMMARY

To recapitulate the results obtained with frog's mesentery:

(1) Quinine acts as an antiphlogistic, but its effect is not sufficient to inhibit entirely the migration of leucocytes even in doses which affect the heart considerably. A dose between 1.25 and 1.56 mgm. per frog can be considered as effective. Smaller doses than 1.25 mgm. have no inhibitory effect.

(2) Atophan acts also as an antiphlogistic. Its effective dose can be stated to be 6.25–12.5 mgm. pro frog. A smaller dose than 12.5 mgm. was ineffective. 12.5 mgm. of atophan inhibited entirely the migration of leucocytes without any depression of the circulation. Even 75 mgm. did not cause any essential weakening of the circulation.

(3) When quinine and atophan are combined in such small doses as  $\frac{1}{3}$  of the maximum ineffective dose they act powerfully, inhibiting the migration of leucocytes, more than much larger doses of quinine alone.

(4) In view of these facts it appears that there exists a synergistic action between quinine and atophan in inhibiting the migration of leucocytes in inflammation of the frog's mesentery.

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# SCIENTIFIC PROCEEDINGS OF THE AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS.

## SEVENTH ANNUAL SESSION

Held at the Harvard Medical School, Boston, December 27, 28  
and 29, 1915

*Edited by the Secretary*

*The Stability of the Growth-Promoting Substance in Butter Fat.* LAFAYETTE B. MENDEL AND THOMAS B. OSBORNE. From the Sheffield Laboratory of Physiological Chemistry in Yale University and the Laboratory of the Connecticut Agricultural Experiment Station, New Haven.

A considerable number of observations are now on record to show that certain mixtures of isolated food substances furnishing a ration upon which animals (albino rat) decline or cease to grow can be converted by the addition of some of the natural fats into a ration adequate for growth. To this category belong butter fat and butter "oil"—the more liquid fraction in which the growth-promoting factor is concentrated.<sup>1</sup> Feeding experiments with both butter fat and butter oil for periods of more than a year under various conditions of temperature, etc., indicate the pronounced stability of the growth-promoting substance as contained in butter fat under ordinary conditions of storage. However, in the butter "oil," in which the growth-promoting factor is more concentrated than in the original fat, gradual deterioration occurred, so that within a year this characteristic potency was eventually almost completely lost.

*The Relation of the Surface Tension of Saponin Solutions to their Hemolytic Activity.* H. E. WOODWARD AND C. L. ALSBERG. From the Bureau of Chemistry, United States Department of Agriculture.

The surface tension of solutions of sapindus, guaiac, quillaja, chlorogallum and agave saponins was determined by Morgan's drop-weight apparatus.<sup>2</sup> It was found that the surface tension value of the saponins in Locke's solution is a little higher than in water, and that in water 100 mgms. of saponin per liter causes nearly the maximum lowering of surface tension, larger amounts up to 1000 mgms. per liter causing

<sup>1</sup> Osborne and Mendel, Jour. Biol. Chem., 1915, xx, 379.

<sup>2</sup> J. Am. Chem. Soc., 1911, xxxii, 349.



only a slight further lowering. The lowest concentration in which these saponins lake erythrocytes was also determined. For chlorogallum, agave and guaiac saponins this could not be done with great exactness since solutions of these saponins agglutinate as well as hemolyze. A comparison of the surface tension values of the solutions of the saponins with their hemolytic power failed to reveal any relation between the surface tension activity and the hemolytic activity of these saponins.

*The Action of Certain Drugs on the Excised Uterus of the Guinea-pig.*

J. D. PILCHER. From the Laboratory of Pharmacology of the University of Nebraska College of Medicine.

The usual methods were used: a longitudinal strip of the uterus (usually pregnant) was immersed in a bath of well oxygenated Tyrode's fluid and the contractions recorded. The fluid extract, the evaporated fluidextract, and the infusion of the drugs were used in the strength of 1-1000, as a rule. There was no difference in the actions of the two alcoholic preparations; with but one exception (figwort) the infusions were inactive. The research took up those ingredients of the popular "female remedies," whose actions have been very little, if at all, investigated. So far some fifteen drugs have been examined; of these, one puts the strip into tonic contractions, four depress it and the rest are practically inactive.

Blue cohosh (*Caulophyllum thalictroides*) in a dilution of 1-1000, and even 1-2000, caused an increase in tone in all cases, with a prompt or often a more gradual decrease in the amplitude of excursion. In about one-third of the experiments the strips remained in tonic contraction above, at or a little below the maximal point attained by the upstroke of the recording lever and remained in tonic contraction from twenty to over sixty minutes.

The following drugs depressed the activity of the strips, arranged in the probable order of their strength: *Pulsatilla pratensis*, Unicorn root (*Aletris farinosa*), Figwort (*Scrofularia marylandica*), Valerian and, to a lesser extent, *Scutellaria lateriflora*. The most marked feature of the depression is a decrease in the amplitude of the excursion; to a less degree, a slowing of the rate. There may be a considerable decrease in amplitude without change in rate but, usually, when the amplitude is greatly decreased the rate is lessened also. At times a 1-1000 solution completely inhibited contractions for several minutes or even much longer. The muscular tone seemed to be the function least affected. This is probably because the strips usually relax completely between contractions; however, a rapid contraction rate may cause an apparent increase in tone because there is not time for relaxation.

The reason for the inactivity of the infusions has been investigated in the case of Valerian only. It is not due to the loss of some volatile principle in the preparation of the infusion, for an infusion made by allowing the drug to stand in water, in a stoppered bottle, at 47°C. for

three or four hours was also inactive. Further the distillate from the fluidextract was inactive while the residue from the same exhibited the usual action. Sodium valerianate, even in a strength of 1-1000 was practically without depressant action; at any rate much less active than the same strength of the fluidextract. Viburnum, which also contains valerianic acid, gave practically negative results. The Oleum valerianae has not as yet been examined.

The following drugs gave negative results: *Viburnum prunifolium* and *opulus*, *Acer spicatum*, *Chamaelirium luteum*, *Senecio aureus*, *Dioscorea villosa*, *Leonurus cardiaca* (motherwort), *Passiflora incarnata* (passion flower) and *Mitchella repens* (squaw vine).

*On the Action of Drugs on the Ureter.* DAVID I. MACHT. From the Laboratory of Pharmacology of the Johns Hopkins Medical School.

1. A ring of pig's ureter suspended in oxygenated Locke's solution at 37°C. begins to contract and relax spontaneously, and these rhythmic contractions continue for many hours.

2. If a pig's ureter be kept on ice in Locke's solution, it can be revived by the above method and will begin to contract spontaneously as late as forty-eight hours after the death of the animal.

3. Precisely the same results were obtained with ureteral rings from a man (nephrectomy).

4. Epinephrin markedly increases the rate of contraction and the tonicity of the ureter.

5. Even a quiescent ureter can be revived and started to beat by the addition of minute doses of epinephrin (one drop 1:10,000 in 50 cc. Locke's solution).

6. Atropin stops the contraction of the ureter.

7. Physostigmin antagonizes the action of atropin.

8. Papaverin causes a marked relaxation or lowering of the tonicity of the ureter, and if not too concentrated does not inhibit the peristaltic contractions.

9. The very low toxicity of papaverin, its general analgesic properties, its local analgesic effect (as noted by Pal and verified by the author), and its property of lowering the tonus of smooth muscle structures suggested its introduction directly into the ureter in a case of ureteral calculus, with a successful therapeutic result, namely the passage of the stone.

10. An extensive study of the pharmacology of the ureter is now in progress.

*The Influence of the Adrenals on the Kidneys.* E. K. MARSHALL, JR., AND DAVID M. DAVIS. From the Laboratory of Pharmacology, and the Brady Urological Institute of the Johns Hopkins Medical School.

Cats, from which both adrenals glands have been removed, die with a urea content of the blood and tissues much higher than that of normal animals. This suggests that renal function is lowered in these animals after adrenalectomy, and that the adrenals exercise some influence on the function of the kidneys.

The adrenals were removed from cats in two operations at intervals of three weeks or longer. These animals lived for from two to eight days. At a time before muscular exhaustion and low blood pressure had appeared, these animals showed a decreased kidney function as measured by the urea content of the blood and excretion of phenol-sulphonephthalein. The nitrogenous metabolism was very little influenced by the removal of the glands, the output of nitrogen in the urine decreasing and waste products accumulating in the blood. In the animals which survived for several days the urea content of the blood rose to a higher level than normal, and remained at this level until just before death when a sharp rise again occurred. Adrenalectomized animals responded differently from normal animals to an injection of a mixture of sodium chloride, urea and creatinin, as measured by the retention in the blood and excretion in the urine. Urea and creatinin are much more slowly eliminated than in normals. The chloride values are variable though not more so than in normals. Water is excreted as well by the operated cats as by normal animals. The blood pressure in normal and adrenalectomized animals may be the same at the time when these changes occur.

*Anaphylatoxin, and the Chemical Theory of Anaphylactic Shock.* RICHARD WEIL. From the Department of Experimental Medicine, Cornell Medical College, New York City.

When antigen and antibody are incubated in vitro, a substance highly toxic to animals is produced ("apotoxin," Richet; "anaphylatoxin," Friedberger). The presence of complement is essential to this process (Friedberger). In order to prove that the same poison is part of the mechanism of anaphylactic shock, Friedberger attempted to show that complement was likewise essential to the production of shock in vivo. This attempt, however, has broken down under criticism. An experiment is here reported in which the heating of antibody at 72° for one-half hour deprives it of its complement-fixing property, while it still sensitizes guinea pigs passively. This fact demonstrates that anaphylactic shock is produced independently of the participation of complement. If this be the case, anaphylatoxin cannot be a part of the mechanism, since this substance is formed only in the presence of complement. The conclusion leads to the rejection of the chemical, or pharmacological, theory of anaphylaxis. This leaves the physical theory, which assumes a disturbance of intracellular equilibrium as the cause of shock. The latter view is accepted on other grounds by Dale, Doerr, and Bayliss.

*The Morphological Changes in the Tissues of the Rabbit as a Result of Reduced Oxidations.* G. H. MARTIN, C. H. BUNTING, AND A. S. LOEVENHART. From the Laboratories of Pharmacology and Pathology of the University of Wisconsin.

The work was done on rabbits, using the apparatus previously described (Kolls and Loevenhart). The animals were kept in an atmos-



phere in which the average oxygen content varied from 5 to 9 per cent, the lowest reading being 3.58 per cent oxygen. Otherwise normal conditions were maintained. Each series was subjected to this procedure for one week, after which the animals were immediately killed, autopsied and the tissues preserved in formalin, Zenker's solution, and alcohol.

Histological changes were manifested in other organs, but the hearts, livers, and kidneys only will be considered here. The extent of the changes varies in the different animals.

In the *heart* the most characteristic and constant changes are: congestion; swollen, granular, vacuolated fibers, some of which show complete disintegration; infiltration of leucocytes, mononuclear cells or fat. Fatty infiltration characterizes most of the hearts. The fibers in some hearts are atrophic.

The *livers* show extensive necrosis in the middle and central zones in some places. The peripheral zone cells are granular and swollen and there are multiple nuclei in many of the cells. There is also serous imbibition of the cells in the central zones. The congestion is very marked on the portal side. The cells in the center of the lobules show fatty infiltration.

The *kidneys* show congestion. The cells show fatty and serous vacuolation. The cells of the convoluted tubules are swollen, congested, and granular. Albuminous precipitation is seen in the lumen of the convoluted tubules, the cells of which in some show hydropic vacuolation.

The general impression obtained is that the cells farthest removed from the blood supply show the more pronounced lesions.

Only such changes which are characteristic of practically all of the specimens are here considered.

*Some Observations on Anaesthesia and Analgesia.* D. E. JACKSON.

From the Laboratory of Pharmacology of Washington University Medical School.

For a number of years past, nitrous oxide has been constantly growing in favor as a general anaesthetic and analgesic. This has been made possible mainly by the introduction of improved methods of administration. Still further improvement in these methods may very well lead to a greatly increased usefulness of the substance. The duration of the anaesthesia under nitrous oxide has also progressively increased from an average of only a minute or two up to an average of perhaps ten minutes or longer. I have been able by an improved method to keep dogs anaesthetized for periods up to five and one-half hours.

Quite recently there has been a slight tendency to avoid the use of nitrous oxide in any prolonged operation (half an hour or more), because it has frequently appeared that the after effects of prolonged nitrous oxide anaesthesia were more deleterious than those of ether. I strongly suspect that this has mainly been due to the use of improper



and unscientific methods of administering the nitrous oxide. I believe also that the cost of nitrous oxide anaesthesia by the method which I have used may be reduced to about 30 or 35 cents per hour for the human subject.

There is a striking similarity between the action of morphin and that of nitrous oxide and oxygen as observed experimentally on dogs. In the present work the nitrous oxide and oxygen have been administered by means of a special apparatus which permits a single "dose" of nitrous oxide to be breathed continuously over and over, without either loss or gain in the amount of the gas, while at the same time oxygen is given to the animal in suitable proportions to maintain the circulation and respiration in good condition. The carbon dioxide and watery vapor exhaled by the animal are constantly removed from the oxygen and nitrous oxide breathed by washing the respiratory medium through strong sodium and calcium hydroxide solution, and through concentrated sulphuric acid respectively. Under these conditions it is possible to administer the nitrous oxide in very gradually increasing doses and the progressive effects produced on the animal can be readily observed and studied.

Among the more striking points of similarity to morphin is the production of Cheyne-Stokes respiration. This is generally present in prolonged anaesthesia in dogs. Presumably this would also be true in man. The conjunctival reflex is not lost in dogs under nitrous oxide. This is true in practically all cases and for all degrees of the anaesthesia. It is only in the presence of immediately impending death from excess of nitrous oxide (which implies a small supply of oxygen, although no excess of  $\text{CO}_2$  may be present in the gases breathed) that a dog will fail to wink its eyelids if the cornea be only gently touched with a piece of cotton. The pupils are dilated, but the light reflex is preserved. The irritability of the cord is much less depressed than is the case with the methane series of anaesthetics. A peculiar feature is noticed in the fact that the animal, while lying quietly and apparently well anaesthetized, may be aroused and waked up by stimulation or shaking in a very similar manner to that possible under a moderate dose of morphin. When thus aroused there is also often observed a marked acceleration and increase in strength of the heartbeat. If the animal be again left alone it will soon return into the somnolent, or perhaps analgesic state, very much as occurs after morphin.

It is difficult to study the analgesic effect of nitrous oxide separate and apart from the production of total unconsciousness in dogs. For these animals, so long as they are conscious, are very likely to struggle and try to escape even though they feel no pain whatsoever.

There seems to be no doubt that, as a rule, the human subject must be more susceptible to nitrous oxide than dogs. In experiments on myself, I have easily been able to produce a state of almost complete unconsciousness with quantities of nitrous oxide very much less than are required for dogs. And similarly the period of time needed to fully anaesthetize a dog is often more than five minutes, while in the human

subject it is seldom more than two minutes (with oxygen). And further, the duration of anaesthesia in dogs, after the face piece is removed, is often not greater than one second, especially if the animal has been anaesthetized for only a little while; in man it may last forty or fifty seconds. A considerable degree of anoxemia is often imperative to keep dogs anaesthetized with the gas. This seems to be much less necessary (even if it cannot always be avoided) in the human subject. I believe this difference in susceptibility is due to the higher organization of the central nervous system in man.

The action of ethyl chloride is about midway between that of nitrous oxide and of ether. Neither nitrous oxide nor ethyl chloride are very suitable anaesthetics for dogs. They are undoubtedly capable of greater usefulness in the human subject. A peculiar walking movement of the legs is very often present in ethyl chloride anaesthesia; spasm of the jaws may occur and may even cause considerable difficulty in breathing. In one animal this clinching of the teeth lasted for several minutes after the drug had been removed (done mainly on account of respiratory difficulty).

The action of ethyl bromide is about midway between that of ether and of chloroform. Spasmodic walking or swimming movements may also occur in dogs under this drug. The corneal reflex is lost early in the administration. The heart and respiration are both strongly depressed by even small amounts (0.5 cc. to 1 cc. in dogs).

*Further Observations on the Anaesthetic Tension of Ether Vapor.* WALTER M. BOOTHBY. From the Surgical Service and Respiration Laboratory of the Peter Bent Brigham Hospital, Boston, Mass.

In previous papers on the anaesthetic tension of ether<sup>1</sup> we assumed that ether vapor was a perfect gas and accordingly calculated its liter weight as 3.3086 grams.<sup>2</sup> But, as is well known, ether vapor is not a perfect gas and its deviation from the gas laws is of the order of about 2.7 per cent. The true liter weight at 0° and 760 mm. is 3.398 grams.<sup>3</sup> For pharmacological and clinical purposes this constant error may be neglected.

On the data presented in our earlier paper, 51 mm. seemed to be

<sup>1</sup> Boothby: The Determination of the Anaesthetic Tension of Ether Vapor in Man, with Some Theoretical Deductions Therefrom as to the Mode of Action of the Common Volatile Anaesthetics. *Jour. Pharm. and Exper. Therap.*, 1914, v, 4.

Boothby and Sandiford: The Calibration of the Waller Gas Balance and the Connell Anaesthetometer. *Jour. Pharm. and Exper. Therap.*, 1914, v, 4.

<sup>2</sup> Landolt, Bornstein and Roth, 1912, p. 141. Liter weight =  $\frac{\text{Mol. Wt.}}{2} \times \frac{1.4292}{16}$

<sup>3</sup> Landolt, Bornstein and Roth, 1912.

Young, p. 151: Density ether at 0° and Sat. Press. 0.000827.

Ramsay and Young, p. 385: Sat. Press. at 0° 184.9 mm.

Therefore at 760 mm. and 0° the liter weight of ether equals

$$1 \times 0.000827 \times \frac{760}{184.9} = 3.398 \text{ gms.}$$

the anaesthetic tension of ether vapor for man. Since then we have had several hundred accurately charted anaesthesias in which we find that after the patients are completely saturated, they can be maintained at 45 or 47 mm. Our previous figure was too high for two reasons: (1) incomplete saturation during the first hour and (2) failure to appreciate that even a lighter degree of anaesthesia was clinically practicable. Under such conditions of even ether administration, any changes in blood pressure and pulse rate are caused by the operative procedure and therefore of valuable aid to the surgeon in determining the extent of the operation. We have had only one case in the entire series in which an idiosyncrasy for ether was shown; in this particular case the blood pressure and pulse rate were dangerously lowered by a tension of 40 mm., necessitating a discontinuance of the ether.

An ether curve, such as is used at the Brigham Hospital surgical clinic, should be made a part of the record of every physiological or pharmacological experiment done under ether anaesthesia as the percentage saturation of the nerve cells affects their reaction to stimulation. It is inaccurate to state that the animal is under 'light' or 'deep' ether anaesthesia. In order to determine the influence of the anaesthetic on the results obtained the ether tension should be recorded throughout the entire experiment as well as at the time of a given observation.

*The Inhibition of the Toxicity of Anesthetics for the Nephropathic Kidney.*<sup>1</sup>

WM. DEB. MACNIDER. From the Laboratory of Pharmacology of the University of North Carolina.

The experimental data have been obtained from experiments conducted in twenty-eight dogs.

The animals were rendered acutely nephropathic by giving uranium nitrate subcutaneously, the dose being 5 mg. per kilogram on two successive days. At the end of this period the animals were rendered partially anesthetic by giving morphin sulphate in the dose of 0.25 cc. of a 4 per cent solution per kilogram. The anterior abdominal wall was anesthetized by a 2 per cent solution of cocain and the bladder was exposed and the urine expressed. The bladder was then returned to the abdomen and the incision closed.

Two animals were employed in each experiment. One of the animals was given intravenously 25 cc. per kilogram of a 3 per cent solution of sodium carbonate, while the other animal which served as a control, was given an equal volume per kilogram of a 0.9 per cent solution of sodium chloride. Both of the animals were anesthetized by Gréhant's anesthetic in 60 per cent strength. The anaesthesia was allowed to persist for two hours and forty-five minutes. At the end of this time any urine which had been formed during the period of anaesthesia was expressed from the bladder and measured. The kidneys were removed for histological study.

<sup>1</sup> The above observations form part of a communication which will appear in *The Journal of Experimental Medicine*, vol. xiii, 2, February, 1916.



1. The animals which received the intravenous injection of the carbonate solution showed in every instance a much greater output of urine during the period of anesthesia than did the animals which received the same volume per kilogram of sodium chloride solution. These control animals (sodium chloride), either became acutely anuric from the anesthetic, or the output of urine, as compared with the output by the carbonate animals, showed a very great reduction.

2. The kidneys of the control animals showed an epithelium which was acutely swollen and in various stages of necrosis. These changes were most pronounced in the convoluted tubules. Accumulations of fat were marked in the loops of Henle.

3. The kidneys of the animals which received the sodium carbonate solution showed an epithelium which gave but slight evidence of injury. There was no necrosis of the epithelium. Fat accumulations in the loops of Henle were slight or absent.

4. In both types of kidneys the vascular pathology consisted in an acute engorgement of the glomerular vessels. There was no histological evidence of degeneration in the glomeruli.

5. The intravenous use of a solution of sodium carbonate protects the kidney acutely nephropathic from uranium against the toxic effect of Gréhan's anesthetic.

6. This protection is associated with the histological preservation of the renal epithelium.

*The Effect of Drugs on Auricular Systole and Their Consequent Effect on Ventricular Efficiency.* CARL J. WIGGERS. From the Department of Physiology, Cornell University Medical College, New York.

The position is taken that the amplitude of the intraventricular pressure curve, optically recorded, represents the most reliable record of ventricular efficiency and that its configuration permits an analysis of the individual factors modifying ventricular efficiency, viz.—(1) the initial intraventricular tension determined (a) by venous volume and pressure (b) by amplitude of auricular contraction and (c) by the  $A_s-V_s$  interval, and (2) the inherent contractile property of ventricular muscle, determined (a) by the length of diastolic rest period preceding ventricular contraction and (b) by chemical modification of properties of contractility and irritability.

Applying this analysis of intraventricular pressure tracings after the use of cardiac depressants and stimulants it was shown by curves that the influence of a drug on the auricle in no case determines the end effect on the ventricle. Thus, atropin decreases ventricular efficiency *in spite* of an increase in amplitude of auricular contraction, an increased initial tension and a more pronounced auricular elevation of intraventricular pressure. This is due to a depression of the contractility probably consequent to the shorter diastolic rest. In short, we have in atropin a drug acting as a stimulant to the auricle and a depressant to the ventricle.



Chloroform by a similar analysis was shown to owe its depressant action on the ventricle to a combined influence of (a) weaker auricular contraction, (b) lower initial tension and direct depression of contractility and irritability.

Pituitrin exerts its depressant action on the ventricle in spite of a high initial tension and more prominent auricular wave and therefore acts directly on heart muscle.

Strychnin shows no influence except in doses inducing convulsions. Such toxic doses increase ventricular efficiency in spite of an unaltered auricular contraction and a lower initial tension.

Epinephrin, when the vagi are cut, increases the amplitude of auricular contraction and augments the auricular wave within the ventricle. This does not determine the increased efficiency found in the ventricle because the initial pressure is decreased. Since the rate is increased, the stimulating action must be exerted through the cardiac muscle or its nerve terminals.

*The Pharmacological Activity of Digitalis Preparations.* JOSEPH H. PRATT AND CONRAD WESSELHOEFT. From the Harvard Medical School.

I. Deterioration with age. Five preparations of digitalis and a tincture of strophanthus were tested by the one-hour frog method in December, 1910, and again in February, 1913, and in December, 1915. They were kept in tightly closed jars and bottles in a dark closet. The digitalis preparations selected were (a) powdered digitalis leaf prepared by Squibb, (b) Merck's powdered digitalis leaf, (c) a tincture made in January, 1910, from Caesar and Loretz's titrated digitalis leaf, (d) a tincture prepared from a fluidextract of digitalis (Parke, Davis and Company), (e) a tincture from digitalis grown in the Rocky Mountains prepared by E. R. Squibb and Sons. The strongest preparation in 1910 was the specimen of Merck's powdered digitalis leaf. Of this the fatal dose was 0.7 mgm. per gram of frog weight. All the preparations, including the strophanthus tincture, lost strength in the five years, varying from 17 to 57 per cent.

II. Moisture in dried digitalis leaves. Nine specimens were tested. The percentage of moisture ranged from 5.8 to 9.1. A newly opened bottle of Caesar and Loretz's titrated leaf contained 5.9 per cent of moisture. The largest amount was found in a newly opened bottle of Merck's powdered leaf. Some digitalis leaf that was received from the importer and labeled "not dried" contained 13 per cent of moisture. The activity of the leaf bore no relation to the percentage of moisture present. In fact the strongest digitalis powder was the one containing the most moisture. This specimen, however, deteriorated more in five years than any other in our series.

III. American digitalis. Two specimens grown in Massachusetts had a very low toxicity. Digitalis from the Rocky Mountains and from Wisconsin was found to be as strong as good German leaves.

*The Influence of Iodin on the Heart.* WILLIAM SALANT AND A. E. LIVINGSTON. From the Pharmacological Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington, D. C.

The experiments were conducted on the isolated frog heart. Iodin dissolved in Ringer's solution containing small quantities of alcohol was perfused for variable periods. The results obtained indicate that even weak solutions of iodine may cause disturbance of heart action. A solution of iodine 1:30,000 produced incomplete cardiac relaxation, irregular action and depressant after-effects. With solutions of 1:15,000 and 1:10,000 the effect was more pronounced, arrest of the heart in diastole being noticed in several instances. The presence of olive oil in the perfusion fluid seemed to decrease the toxicity of iodine. Experiments with sodium iodide showed that this salt is much less toxic than a corresponding amount of iodine alone.

*The Absorption and Elimination of Different Dyes.* WILLIAM SALANT AND ROBERT BENGIS. From the Pharmacological Laboratory, Bureau of Chemistry, Department of Agriculture, Washington, D. C.

Water-soluble and fat-soluble coal tar dyes are eliminated in the urine and in the bile. The former were administered intravenously, subcutaneously and injected into the small intestine. In every case they appeared in the bile before they were eliminated in the urine. After introduction into the small intestine of the rabbit the bile became deeply colored in 12 to 16 minutes, while they appeared in the urine within 2 to 3 hours. When injected intravenously they appeared in the bile within 5 to 6 minutes, in the urine in 20 to 30 minutes. In experiments on dogs which received erythrosin by vein, the urine was free from dye 2 hours after its administration, but the bile was deeply colored. Elimination was slow. After 60 mgs. erythrosin per kilo were given to one rabbit intravenously, the urine was colored for a period of 11 days. In another experiment 0.25 to 0.4 per kilo injected subcutaneously were eliminated in 14 days.

The elimination of the fat soluble dyes is very slow. Fat soluble dyes were in most cases given subcutaneously. In a few experiments they were injected into the peritoneal cavity, rabbits being used in all these tests. Elimination in the urine was observed within 2 to 5 hours in some experiments, but the urine in other cases was free from dye at this time. In no case was lipuria present. Half to one gram per kilo, given subcutaneously, were eliminated in 60 days in the urine. Elimination in the bile ceased, while the urine free from fat still contained the dye. The product when isolated from the urine, gave a different melting point and was soluble in water. Evidence of conjugation with glycuronic acid was obtained.

The elimination of fat-soluble, as well as water-soluble dyes, in the urine may be inhibited in case of injury to the kidneys. The liver assumes the function of elimination in this case. The intravenous injection of zinc into rabbits inhibited the elimination of erythrosin in

the urine, but it was found in the bile, which was obtained several days later. Oil of chenopodium, if given several days after the administration of a fat-soluble dye, may inhibit its elimination, but has no effect when given at the same time with the dye.

*The Detoxifying Action of Sodium Salts upon Potassium Salts on Intravenous Injection.* S. AMBERG AND H. F. HELMHOLZ. From the Otho S. A. Sprague Memorial Institute, Chicago, Ill.

Guinea pigs of about 200 grams weight are killed as a rule on intravenous injection of 2 cc. of a 1 per cent KCl solution, or of 2 cc. of a 1.75 per cent  $K_2SO_4$  solution given at the rate of 1 cc. per minute. Sometimes it may happen that animals survive an intravenous injection of even 2 cc. of a 1.5 per cent KCl solution. A 1.5 per cent KCl solution was used in our experiments. All animals used in a day's series were from a single source and daily control experiments were made with the potassium solutions alone because of the varying susceptibility of different strains. Solutions were prepared containing 3, 5 and 10 per cent NaCl and 1.5 per cent KCl. A number of animals survived the injection of 4 and 5 cc. of these mixtures. Preceding the injection of 1.5 per cent KCl solution by 5 cc. 5 per cent NaCl, the animals survived 3 cc. of the KCl solution. When the KCl injection followed one of 5 cc. 0.55 per cent NaCl the animals died. On injection of 2 cc. of a mixture containing 6 per cent anhydrous  $Na_2SO_4$  and 1.5 per cent KCl, five animals out of ten survived, that is the detoxifying effect of  $Na_2SO_4$  was not as marked as that of NaCl. Experiments with mixtures containing 11.7 per cent sodium acetate and 1.5 per cent KCl did not show any detoxifying effect of the sodium acetate. Experiments with mixtures containing 5 per cent NaCl and 1.75 per cent  $K_2SO_4$  showed that animals may survive an injection of 4 cc., and animals having received 5 cc. 5 per cent NaCl previous to the injection of 2 cc. 1.75 per cent  $K_2SO_4$  also survived. A series of experiments was conducted with  $NH_4Cl$  in place of the potassium salts. The fatal dose of  $NH_4Cl$  was found to be 2 cc. of a 2 per cent solution. Only two of eight animals receiving a mixture containing 5 per cent NaCl and 2 per cent  $NH_4Cl$  survived. When the injection of 2 cc. 2 per cent  $NH_4Cl$  was preceded by one of 5 cc. 5 per cent NaCl some animals recovered from a severe intoxication.

*On the Toxicity of Various Commercial Preparations of Emetin Hydrochloride.* R. L. LEVY, M.D. AND L. G. ROWNTREE, M.D. From the Medical Clinic of the Johns Hopkins Hospital, Baltimore.

The widespread use of emetin hydrochloride in the treatment of amebic dysentery and of pyorrhoea alveolaris makes more precise knowledge of the toxicity of the commercial preparations employed desirable. Two cases occurring in the Medical Service during the past year forcibly emphasize this fact.

The first case was that of a syphilitic man of 56, who, because of a diarrhoea, supposedly amebic in origin, was given hypodermically 29



grains of the drug during a period of twenty days. He died ten days after discontinuing the emetin treatment, with evidences of acute renal insufficiency accompanied by acidosis. At necropsy, syphilitic aortitis, chronic indurative colitis and bronchopneumonia were the only findings of importance.

In the second case, 2 grains were given hypodermically, during a 4-day period, to an undernourished, anemic woman who had marked pyorrhoea and gingivitis. A severe diarrhoea developed, with pus and blood in the stools, followed by a toxic delirious psychosis. On discontinuing the emetin, recovery ensued.

A dog was injected subcutaneously with some of the same preparation employed in Case II, and died with a hemorrhagic gastro-enteritis, after three doses of 10 mgms. each, given on successive days.

Subsequently, studies were made on 62 animals, the series including dogs, cats and rabbits. Five commercial preparations were investigated: Burroughs, Wellcome and Company (ampoules); Eli Lilly and Company (ampoules); Merck and Company (crystals); Parke, Davis and Company (ampoules); and Sharp and Dohme (hypodermic tablets). Both subcutaneous and intravenous injections were made. The following facts were brought out:

1. Various commercial preparations differ widely in toxicity. In dogs, for instance, a total dosage of 3 mgms. per kilogram of the most toxic preparation given subcutaneously killed in three days, whereas, 19 mgms. per kilogram of another preparation required eleven days. In cats, 14 mgms. per kilogram (total dosage) caused death in three days, whereas, 29 mgms. of another killed only after seven days. For immediate death following intravenous administration, 4 mgms. per kilogram in one instance, 18 mgms. per kilogram in another, were necessary. The therapeutic dose (mgms. per kilogram) closely approximates that necessary to produce toxic symptoms.

2. When injected intravenously, emetin is a powerful cardiac poison, causing at times fibrillation of the ventricles, from which the animals may recover. It is also a circulatory and respiratory depressant.

3. In fatally poisoned dogs, the characteristic lesion is a hemorrhagic gastro-enteritis. Lesions in cats and rabbits are slight and inconstant.

4. The factors of blood coagulation are disturbed in poisoned animals.

5. There is found no evidence of renal insufficiency; a slight terminal acidosis is present.

On reviewing the reported cases in which ill effects have followed the clinical use of emetin, it is apparent that diarrhoea is an early toxic manifestation and that peripheral neuritis is one of the most frequently observed sequelae, even after the administration of therapeutic doses.

As a result of these observations, it is suggested that emetin preparations be cautiously employed. It is desirable that the drug be given subcutaneously, in courses, at intervals of several days or a week. One-third grain three times a day for a week or ten days is usually a



safe dosage in amebic infections; one-half grain daily for from three to six days suffices, according to Bass and Johns, in pyorrhoea. Large doses should be avoided. Intravenous injections should be employed only in extreme cases. If this mode of administration seems imperative, small doses, well diluted (one-half grain in 100 cc. salt solution) should be slowly given, and the blood pressure should be carefully observed during the injection.

*The Comparative Action of the Chief Alkaloids of Cinchona.* WORTH HALE. From the Laboratory of Pharmacology of the Harvard Medical School.

In 1914 a report was made of work done in comparing the action of cinchonin, cinchonidin and quinidin with that of quinin on the isolated uterus. All possessed marked stimulating effects without subsequent paralysis in the amounts used.

This work has been extended to experiments upon the uterus of the living animal under paraldehyde anaesthesia and curare. The cats were submersed in a bath of 0.9 per cent sodium chloride at 37° to protect the uterus from air currents and chilling.

Quinidin appeared to be more active than quinin as were also cinchonin and cinchonidin, thus confirming the work on the isolated uterus. The exact ratio of activities to that of quinin was not worked out definitely but using 100 as representing the activity of quinidin, cinchonin had a value of about 60 and cinchonidin and quinin about 30. These figures correspond, therefore, in a general way to those obtained on the isolated organ.

All four alkaloids induce a marked fall in blood pressure in doses of 1 to 6 mgms. per kilogram of cat, but this fall in pressure is less marked if the drugs are injected into the jugular vein slowly and diluted with several cubic centimeters of normal saline.

*Further Studies on the Pharmacological Action of Oil of Chenopodium.*

WILLIAM SALANT AND A. E. LIVINGSTON. From the Pharmacological Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington, D. C.

Oil of chenopodium produces marked depression of the circulation and respiration when introduced into the stomach or small intestine. Five cubic centimeters of oil of chenopodium emulsified with acacia and sodium carbonate and injected into the duodenum in a cat was followed immediately by a fall of blood pressure amounting to 20 mm. Hg or about 18 per cent. Thirteen minutes later the blood pressure suffered another decrease of 50 mm., Hg while respiration was now 9 per minute instead of the original 18 per minute. Blood pressure sank to 22 mm. Hg (from 145) within two minutes, and apnea occurred within three minutes. Exceptionally, however, no change either in respiration or circulation could be noticed for a considerable length of time. Absorption from the stomach was very slow, as no change was observed after 1½ to 1¾ hours after its introduction. Rabbits behaved like cats

when oil of chenopodium was introduced into the stomach or into the duodenum, but in dogs absorption was also very slow when it was introduced into the duodenum and no effects could be observed after 1½ hours.

Experiments on the effects of drugs in poisoning with oil of chenopodium in the intact animal indicate that caffeine may stimulate respiration provided the poisoning is not severe. In the latter case it sometimes aids in the production of apnea. No effect on the blood pressure could be observed, although transitory increase in the heart rate was caused by caffeine under these conditions.

In experiments with oil of chenopodium on the isolated frog heart we obtained marked cardiac depression in all cases in which this substance or ascaridol was perfused in the later stages of the experiment, but this was not always the case in the fresh heart. After a number of perfusions were made with Ringer's solution containing but a few drops of oil of chenopodium or ascaridol, the heart could be brought to a standstill. In most of our experiments a small quantity of olive oil was added to Ringer's solution and a fine emulsion was obtained. When three minimis oil of chenopodium were added to 100 cc. of this emulsion a noticeable depression could be produced even in the fresh heart by perfusing for 1 to 1½ minutes. Arrest of the heart in diastole was observed when perfusion lasted 3 to 4 minutes, but recovery frequently took place although considerable time usually elapsed. The effect of cardiac stimulants either when perfused in combination with oil of chenopodium or after the heart was subjected to its action was studied. Caffeine 0.1 per cent to 0.2 per cent with one minim oil of chenopodium to 100 cubic centimeters Ringer's solution produced much greater cardiac depression than oil of chenopodium alone. Adrenalin in concentration 1:200,000 to 1:25,000 on the other hand was found to exert a very powerful antagonistic action to oil of chenopodium. When perfused with oil of chenopodium the heart action was stimulated to a marked degree, no aftereffect being observed. In some experiments perfusion with adrenalin revived the heart whose activity was abolished by oil of chenopodium.

Similar results were obtained with digitalis. Concentration of 1:1000 to 1:100 of the tincture perfused with oil of chenopodium almost completely overcame its depressing effect, especially when the stronger solutions of the tincture were used. A very marked stimulating action could be observed when the perfusion with the mixture was discontinued, the effect often lasting 5 to 7 minutes. In several experiments perfusion with oil of chenopodium was continued until the heart stopped, but contractions returned soon after perfusion with digitalis was begun.

*The Fate of Sodium Citrate in the Body.* WILLIAM SALANT AND LOUIS E. WISE. From the Pharmacological Laboratory, Bureau of Chemistry, United States Department of Agriculture, Washington, D. C. Sodium citrate, injected intravenously in different animals, disappears very rapidly from the circulation. After 22 to 50 minutes a

faint trace of it could be detected in the blood. When a second or third dose was given its disappearance was much slower. After the subcutaneous injection of sodium citrate, it appeared in the blood within 15 minutes and increased, reaching a maximum a half hour after injection. Blood taken  $1\frac{1}{2}$  to  $2\frac{1}{2}$  hours later showed the same amount of citrate. When sodium citrate was fed by mouth in doses of 3 grams per kilo, small amounts only could be detected in the blood, approximately 15 to 20 mgms. per 100 cc. blood within 2 to 3 hours.

The oxidation of sodium citrate was studied in different animals. After its subcutaneous injection in rabbits an average of 12 per cent was recovered in the urine. Twenty-five to 28 per cent was in the urine of two rabbits, but in eleven others the amounts varied between 4.1 to 14 per cent. Experiments on the effects of hemorrhage failed to show any deviation from the normal. An average of 12 per cent of the amount administered subcutaneously was also found in the urine of these rabbits. About 25 per cent of blood was drawn in 24 hours in these experiments. Oxidation of sodium citrate in carnivorous animals is much slower. The average amount recovered in cats was 30 per cent, and in one experiment on a dog, 40 per cent of the amount administered was obtained.

*Paraoxyphenylethylamin as a Morphin Antagonist.* (Preliminary communication.) H. G. BARBOUR, L. L. MAURER AND W. C. V. GLAHN. From the Department of Pharmacology, Yale University School of Medicine.

Paraoxyphenylethylamin, tyramin,  $\text{OH} \cdot \text{C}_6\text{H}_4 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH}_2$ , is capable, within certain limits, of antagonising the respiratory depression produced by morphin in the rat and in man.

The respiratory volume of the *rat* is diminished by 60 per cent or more after subcutaneous doses of morphin ranging from 0.5 to 2.0 mgms. per 100 gram animal. Paraoxyphenylethylamin in doses of 1 to 10 mgms. per 100 gram rat causes an increase in volume and rate. Larger amounts may depress the respiration.

In a number of experiments both drugs were injected in the same solution. Doses of morphin varying from 0.9 to 1.8 mgms. (per 100 gram rat) were given with 1.6 to 5.8 mgms. (per 100 gram rat) of paraoxyphenylethylamin. The average volume fall in 22 experiments was only 35 per cent. If the morphin was increased to 2 mgms. (per 100 gram rat) the antagonism became less evident, but if instead we increased the *p. oxyphenylethylamin* to 20 mgms. (per 100 gram rat) the fall in respiratory volume was reduced to an average of 15 per cent.

When the two substances were injected on opposite sides of the animal the morphin effect became at first rather marked, the respiration falling to an average of 44 per cent below normal. A return to or above normal however occurred within 1 to 2 hours after the injection. This recovery was apparently permanent. Where morphin is given alone in similar doses there is usually no return to normal within 5 or 6 hours, often much longer. The antagonism cannot therefore be ascribed to delayed absorption of morphin.



In a number of experiments where both drugs were given the response of rats to pain stimuli was clearly diminished during a considerable period of normal respiratory volume.

If the drugs are given at different times within one-half hour of each other the antagonism is still seen.

The tolerance factor was excluded by using fresh rats.

Rats could not be made tolerant of minimal lethal doses of morphin by simultaneous injection of paraoxyphenylethylamin.

In *man* the antagonism has been successfully shown in five-hour experiments in three different individuals.

Morphin sulphate subcutaneously in 16 mgms. doses in two of us and 10 mgms. in the other gave consistently a marked fall in volume and rate of respiration. The respiratory exchange was also diminished. The alveolar  $\text{CO}_2$  was increased.

Thirty mgms. of paraoxyphenylethylamin hydrochloride subcutaneously in a 66 kilo man gave an increase in respiratory rate and volume, also in produced  $\text{CO}_2$  and absorbed  $\text{O}_2$ . There was no change in alveolar  $\text{CO}_2$ .

Twenty-five mgms. of paraoxyphenylethylamin given to a man of 80 kilos gave an increase in rate and volume of respiration and in the respiratory exchange followed within an hour by a secondary decrease in all of these. The alveolar  $\text{CO}_2$  was unchanged.

In all three of us 16 mgms. of morphin failed to produce the effects above described when given simultaneously with 40 or 50 mgms. of paraoxyphenylethylamin. In one of these cases the alveolar  $\text{CO}_2$  was increased, but in none were the volume, rate, or metabolism lowered. These in fact underwent a marked increase in one case.

The antagonism seems therefore to be due to the fact that paraoxyphenylethylamin is a powerful indirect stimulant of the respiration.

No rise of blood pressure greater than 25 mm. occurred.

The tolerance factor was eliminated by giving the combined drugs in two of the subjects previous to the days on which morphin was given alone.



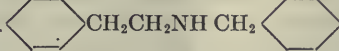

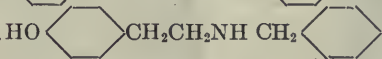

Other morphin effects were definitely present after the two drugs had been given together, although the respiration was not diminished. There was marked dryness of the mouth, and constriction of the pupils as well as late vomiting in the two individuals who showed this idiosyncrasy with morphin alone. A "state of abstraction" was noted, and pain sense was somewhat diminished.

These subjective phenomena of morphin action were probably somewhat less marked when the paraoxyphenylethylamin was given.



*Action of some Derivatives of Phenylethylamin.* H. G. BARBOUR. From the Department of Pharmacology, Yale University School of Medicine.

Comparative studies of the following substances have been made:

- I. Phenylethylamin.....  
 II. *p.* Oxyphenylethylamin.....HO  
 III. Phenylethylbenzylamin.....  
 IV. Phenylethylmethoxybenzylamin...  
 V. *p.* Oxyphenylethylbenzylamin.....HO  
 VI. Phenylethyl-*p.* oxybenzylamin....

The hydrochlorides of all of these substances were employed. With the exception of *p.*oxyphenylethylamin they were kindly furnished by Prof. Treat B. Johnson who was the first to synthesize three of them, viz: phenylethylbenzylamin, phenylethylmethoxybenzylamin and phenylethyl-*p.*oxybenzylamin.

*p.*Oxyphenylethylamin is by far the least toxic of the series, for the minimal lethal doses for 20-gram white mice were respectively: 6 mgms., 55 mgms., 5 mgms., 6 mgms., 12.5 mgms. and 10 mgms.

Similarly, the smallest dose which slowed the heart with lowering of the blood pressure when given intravenously in decerebrate curarized cats, were respectively: 20 mgms., > 60 mgms., 10 mgms., 20 mgms. and 30 mgms. *p.*Oxyphenylethylamin given to such cats in doses up to 60 mgms. gave only augmentation and acceleration of the heart.

The respiratory rate of mice was stimulated by one or more milligrams of all of these substances except V, 1 to 5 mgms. of which slowed the respiration, 10 to 12.5 mgms. accelerating it.

The body temperature of a 5-kilo dog was appreciably raised by 50 mgms. of I or of II but a similar amount of III and IV was without effect, as was 20 mgms. of V.

"*Sympathomimetic Actions.*" Definite increases in the blood pressure of decerebrate curarized cats were obtained from 0.01 mgm. of either phenylethylamin or *p.*oxyphenylethylamin, but not with smaller doses. The latter substance in optimum dosage is capable of two or three times as great a rise in blood pressure as the former. One mgm. of either III or IV or 5 mgms. of VI was required for a demonstrable pressor effect. Larger doses of the last three never gave increases of over 5 to 10 mm. Hg. V did not affect the blood pressure.

The intact uterus of non-pregnant cats was inhibited by all except V. The minimal effective doses were respectively 10 mgms., 0.25 mgms., 5 mgms., 10 mgms., none, 5 mgms. All six of the substances relax the isolated non-pregnant guinea-pig uterus.

I and II dilated the frog pupil; III and IV constricted it, but not constantly; V gave no effect; VI gave dilatation but not constantly.

Exophthalmos, a phenomenon said to be indicative of "sympathomimetic" action, is quite persistent in the mouse after one or more milligrams of *p*-oxyphenylethylamin. The other substances studied, except V, all gave this effect but it was less marked and of shorter duration.

A definite but rather weak affinity for the sympathetic nervous system is therefore exhibited by the hydrochlorides of the secondary amines, phenylethylbenzylamin, phenylethylmethoxybenzylamin, and phenylethylparaoxybenzylamin.

Mr. L. H. Nachamofsky and Mr. A. M. Hjort have rendered valuable assistance in this work.

*The Influence of Temperature on the Onset of Strychnin Convulsions in the Intact Frog.* BENJ. H. SCHLOMOVITZ AND C. S. CHASE. From the Department of Pharmacology, State University of Iowa.

The tetanus produced in the intact frog, *rana pipiens*, by injecting into the dorsal lymph sac strychnin sulphate (0.17 mgm. per gram frog) occurs in increasingly shorter intervals with the increase of temperature, in the range of 1° to 36°C. The internal temperature of the frog was taken by means of a thermometer thrust through the mouth and into the gullet of the animal, immediately before the injection, and immediately after the first convulsion, and the temperature of the animal throughout the procedure was taken as the average of the two determinations. The frogs were in good condition being prepared for the winter season, and having sufficient food. Maximal doses of strychnin were given, (a) so that the percentage of strychnin eliminated, diverted, or destroyed in the tissues compared to the total amount circulating in the system would be negligible; (b) so that the experiments could be completed in a period with the same observer attending throughout; (c) so that the large mass of the strychnin would preclude all other actions except tetanus in time period; and (d) so that a sufficient amount would be present in the dorsal lymph sac reducing thereby the factor of varied absorptive powers of individual frogs to a minimum. No attempt is made at present to explain the influence of temperature on various factors—circulation, permeability of membranes, dissociation of the salt, etc. Knowing the temperature of the frog, the time between injection and first convulsion is predictable within definite limits, by looking up the average at that temperature in our table and making a range of possibility above and below the average by means of the average standard deviation which is about 30 per cent at practically all temperatures. The average length of response at 2°C. is 565 seconds, and at 36°C. it is 34 seconds. The coefficient *Q* expressing the decrease in time or velocity of reaction with 10°C. difference for the occurrence of tetanus is 2.375 (average). The empirical formula used was

$$\left(\frac{K_1}{K_0}\right)^{\frac{10}{t_1-t_0}} = Q_{10}$$

*Further Observations on the Clinical Actions of Veratrum.* R. J. COLLINS. From the Pharmacological Laboratory of the Western Reserve University Medical School.

The effects of veratrum on the circulatory system have been studied in a further series of cases in the same manner as reported in a previous publication.<sup>1</sup> These comprised normal and pathological individuals, among which were the following: alcoholic cirrhosis, paroxysmal tachycardia, heart block, acute and chronic nephritis. The following conclusions seem justified.

1. A slowing of the pulse and fall in blood pressure occurs with single therapeutic doses of 15 to 30 minims of the tincture veratrum album, and this occurs independently of such symptoms as nausea and vomiting (toxicity).

2. Large and repeated doses produced a slowing of the pulse in 100 per cent of the cases; a fall in both the systolic and diastolic blood pressure in 73 per cent. The fall in blood pressure is roughly proportional to the dose.

3. Repeated small or large doses with short intervals between doses give rise to symptoms of toxicity (headache, nausea, vomiting, etc.). These effects are absent with small doses given at longer intervals.

4. To both therapeutic and toxic doses normal individuals respond with a fall in systolic and diastolic blood pressure and a slowing of the pulse.

5. Very little or no effect was observed in the following conditions: arteriosclerosis, paroxysmal tachycardia, heart block (one case of each).

6. The most marked effects with veratrum are observed in cases of hypertonus.

7. Using tincture of gentian under the same conditions as veratrum in the same individuals, no effects on the circulation were demonstrable.

*Some Observations on the Elimination of Hexamethylenetetramin (Urotropin).* K. G. FALK AND K. SUGIURA. From the Roosevelt Hospital, New York City.

A method for the estimation of hexamethylenetetramin was described.

In general terms, the percentage amounted excreted in the urine after administration by mouth in presumably normal individuals was greater the lower the specific gravity of the urine.

The concentration of formaldehyde in the urine had no apparent connection with the amount of hexamethylenetetramin excreted.

In a number of pathological cases involving impairment of kidney function, abnormally small amounts of hexamethylenetetramin were excreted.

<sup>1</sup> Collins, R. J., Arch. Int. Med., xvi, pp. 54-58.



*Excretion of Salicyl in the Urines of Rheumatic and Non-Rheumatic Individuals.* R. W. SCOTT, T. W. THOBURN AND P. J. HANZLIK.

From the Medical Clinic, City Hospital, and the Pharmacological Laboratory, Western Reserve University.

Various explanations have been offered for the striking action of salicylates in acute articular rheumatism. None of these, however, have been satisfactorily established. We have investigated the subject along several lines, but are not prepared to support any explanation. The object of this contribution is to compare the quantitative excretion of salicylates in rheumatic and non-rheumatic, or practically normal, subjects. This was intended to show whether the therapeutic effects of salicylates could be based on differences in the retention or "affinity" of the normal and rheumatic organism for salicyl.

The work was conducted in a quantitative manner as much as possible.

Certain phases of the work still remain uncompleted, but a study of the data at hand seems to justify the following conclusions.

1. The total excretion of salicyl in urine is about 10 per cent less in rheumatic than normal individuals.

2. This difference is greatest in the early periods, that is, during the first ten to twenty hours after administration of the salicylate.

3. The concentration of salicyl in the blood of rheumatic individuals at "toxicity" (about the end of first ten hours) is less (almost one-half) than in normal individuals. The percentage concentration of the urine at the end of this time is also less in rheumatic than in normal individuals.

4. These differences are not due to a difference in diuresis, for in each case the urine was collected until salicyl-free, and therefore these are not due to incomplete collection. The diuresis during the early periods was practically the same in both types of individuals.

5. This leaves retention, vicarious excretion and destruction as possible explanations of these differences. As to retention the data indicated that there is none.

6. There is either destruction or vicarious excretion in the early periods. Unfortunately, as to excretion by channels other than urine and feces no data were obtained. The total feces showed only a trace or no salicylate in each case. Excretion by the sweat might explain, in part at least, the loss of salicyl. In this connection, however, increased destruction of the salicyl in the febrile rheumatic organism must not be overlooked.



*Salicyl in Blood and Joint Fluid of Individuals Receiving Full Therapeutic Doses of Salicylate.* R. W. SCOTT, T. W. THOBURN AND P. J. HANZLIK. From the Medical Clinic, City Hospital, and the Pharmacological Laboratory, Medical School, Western Reserve University.

The percentage concentration of salicyl in the blood and joint fluid of rheumatic individuals receiving full therapeutic doses of salicylate is approximately the same.

There is no demonstrable free salicylic acid in the joint fluid of individuals suffering with rheumatic fever.

*Salicyluric Acid.* P. J. HANZLIK. From the Pharmacological Laboratory, Medical School, Western Reserve University.

The methods which have been hitherto used for isolating what was supposed to be salicyluric acid, did not yield, when applied to human salicyl urines, any notable quantity of any pure product. The products that are obtained are not well characterized, do not have any distinctive properties, and apparently do not contain glycocholic acid.

From this, it appears improbable that salicylates are converted into salicyluric acid in the human organism. The products that have been interpreted as salicyluric acid were presumably more or less impure salicylic acid, that had been imperfectly separated in the process of isolation.

*Colorimetric Method for the Estimation of Free Formaldehyde.* R. J. COLLINS AND P. J. HANZLIK. From the Pharmacological Laboratory, Medical School, Western Reserve University.

A method for the colorimetric estimation of formaldehyde has been devised in which the phloroglucin reagent is used.

Permanent color standards which match the phloroglucin in different concentrations of formaldehyde can be made from mixtures of 0.025 per cent congo red and 0.01 per cent methyl orange.

These mixtures have been experimentally determined for a considerable number of concentrations of formaldehyde between 1:1,000,000 and 1:30,000.

The colorimetric method is more accurate than the Romijn, U. S. P. and hydroxide-pressure methods.

The colorimetric method possesses an important advantage over other methods in that it is directly applicable to urine for the determination of free formaldehyde.

*Further Studies on the Rôle of the Liver in Acute Polycythaemia.* PAUL DUDLEY LAMSON. From the Pharmacological Laboratory of the Johns Hopkins University.

The work of a previous paper by the present author was reviewed,<sup>1</sup> in which it was proved that the liver is the organ where the process of

<sup>1</sup> P. D. Lamson. The Rôle of the Liver in Acute Polycythaemia: A Mechanism for the Regulation of the Red Corpuscle Content of the Blood. *Journ. of Pharm. and Exper. Ther.*, 1915, vii, 169.

increasing the red corpuscle content of the blood in acute polycythaemia takes place, and where it was shown that shutting off the arterial blood supply to the liver excludes this polycythaemia. Further control experiments were reported in which all possible anastomoses were excluded, and the success of depriving the liver of its arterial blood supply proven by dye injections. These experiments show conclusively that when the arterial blood supply to the liver is shut off, no polycythaemia takes place on the injection of epinephrin.

As to the processes in the liver by which this increase in the number of red cells is brought about, the following points have been established.

1. The presence of newly formed red cells has not been observed.
2. An increased arterial blood pressure is not necessary for the production of polycythaemia.
3. No increase in the number of red cells takes place after the injection of epinephrin if the arterial blood supply to the liver has previously been cut off. Later return of the normal arterial blood supply to the liver causes a marked increase in the number of red cells without further injection of epinephrin.

4. Control clamping of the hepatic artery with no injection of epinephrin causes no increase in the number of red cells.

5. The injection of epinephrin causes a definite decrease in the plasma and total blood volumes.

6. Dr. Keith and I have shown that this decrease in blood volume takes place equally well when the hepatic artery is ligated.

From these facts I have reached the following conclusions in regards the mechanism in the liver by which polycythaemia is produced.

During epinephrin polycythaemia the plasma volume is decreased, but not sufficiently to account entirely for the increase in number of red cells. When the hepatic artery is ligated epinephrin causes the customary decrease in blood volume, but no increase in the number of red cells. Such a condition can not occur without a destruction of red cells having taken place. No evidence of such a rapid destruction has been observed. On the other hand, later opening of the hepatic artery causes the red cells to increase markedly. As control experiments of clamping the hepatic artery without injection of epinephrin show no increase in the red count on removal of the clamp, the evidence is very much in favor of a storing of red cells having taken place when epinephrin is injected with the hepatic artery ligated.

Acute polycythaemia appears then to be due partly to a decrease in the plasma volume, and partly to a washing out of corpuscles normally stored or recently deposited in the liver, and not to a new formation of red cells.

*Further Studies in Nicotin Tolerance.* C. W. EDMUNDS AND M. I. SMITH. From the Pharmacological Laboratory of the University of Michigan, Ann Arbor.

In a paper<sup>1</sup> published some years ago one of us (E.), showed that dogs could be rendered tolerant to nicotin by the repeated injection of large

<sup>1</sup> Journ. Pharm. and Exp. Therap., 1909, i, 27.

doses of the alkaloid, but that this tolerance was slight and gained only with great difficulty. Later Dixon and Lee<sup>2</sup> having treated rabbits with nicotin, made emulsions of the livers of these animals, and to each of these added a small amount of the alkaloid. They placed the emulsions for a time in an incubator and then compared the amount of nicotin remaining in each emulsion made from the tolerant animals' liver with that made from a normal liver similarly treated. They found that in a majority of cases the liver from the tolerant animal apparently destroyed a larger amount of the alkaloid than did the normal liver. In three animals this was not true. They conclude that tolerance to this substance is gained by the formation in the liver of a ferment which destroys the poison.

Inasmuch as this fact had apparently been shown to be true for the rabbit, it seemed that it might be interesting to see whether the same might hold for dogs. Accordingly ten dogs were rendered tolerant in the same manner as has been described in the earlier paper referred to. When the process was complete the animals were killed and the ground liver incubated with a definite amount of nicotin. Normal livers as controls were treated in the same manner. The amount of nicotin in each case was estimated upon cats by the blood pressure method. The results showed that in four animals there was no difference in nicotin-destroying power between the injected animal and the normal. In three, the liver from the tolerant destroyed more than did the normal, but this was offset by the remaining three of the ten dogs in which the controls were more active than the the tolerant livers.

It seemed clear from these negative results that in the dog at least, the livers of all animals destroy a certain amount of nicotin but that they vary considerably in activity in the respect. It is possible this property may be developed by treatment, but our experiments do not furnish any support for this theory.

*The Effects of the Prolonged Feeding of Aluminium Salts.* GEORGE B. ROTH AND CARL VOEGTLIN. From the Division of Pharmacology, Hygienic Laboratory, United States Public Health Service.

When aluminium in the form of aluminium lactate or sodium aluminium lactate, is fed to rabbits, cats, or dogs for long periods of time, certain distinct effects are produced. Rabbits which received by stomach tube the equivalent of 20 mgm. of aluminium per day, died after about two months. The only symptom which they manifested was diarrhoea. At autopsy corrosion of the stomach, together with congestion and a hemorrhagic condition of the intestinal mucosa were found. If the daily doses were increased to 80 mgm. there usually resulted besides diarrhoea some loss in weight and certain urinary changes, namely albuminuria accompanied by many hyaline and granular casts. At autopsy the animals which received 80 mgm. per day showed numerous

<sup>2</sup> Quart. Jour. Exp. Phys., 1912, v, 373.



areas of intestinal hemorrhages and a few areas of ulceration which were especially marked in the large intestine.

Cats which received aluminium lactate as a 10 per cent aqueous solution by stomach tube in daily doses varying from 40 to 80 mgm. died after from 3 to 17 days. When given in this way, vomiting occurred on the first or second day and continued at irregular intervals until the death of the animal. The desire for food while not wholly lost was greatly decreased and all the animals lost in weight. Shortly before death albuminuria appeared. At autopsy varying degrees of corrosion of the stomach mucosa and congestion of the upper part of the small intestines, were generally found, while in some animals the spleen, liver and kidneys were also congested. Two cats that were given aluminium lactate in milk in 20 to 40 mgm. doses for a period of about eight weeks, seldom vomited after receiving the mixture and showed alternating periods of diarrhoea and constipation. At the end of seven weeks the animal which received the 40 mgm. dose developed a slight albuminuria and at autopsy showed changes similar to those occurring in dogs.

In dogs daily doses of 20 mgm. of aluminium lactate for periods of four months produced diarrhoea only. At autopsy, however, congestion and scattered hemorrhages were found in the stomach, while the intestines showed not only congestion but many areas of minute hemorrhages and thickening of the intestinal mucosa.

*Is the Dilatation of the Pupil Following Gangliectomy Due to Vaso-dilatation?* T. S. GITHENS AND S. J. MELTZER. From the Department of Physiology and Pharmacology of the Rockefeller Institute for Medical Research.

Intravenous injection of adrenalin causes a dilatation of the pupil which always disappears in less than a minute. Subcutaneous injection or instillation of a few drops into the conjunctival sac produces no change in the size of the pupil. Some thirteen years ago S. J. Meltzer and Clara Meltzer discovered that, about twenty-four hours after removal of the superior cervical ganglion in rabbits, intravenous injection or a single installation of adrenalin is capable of causing a maximal dilatation of the pupil which may last for several hours. One of us (M.) found later that the same holds true for the cat when the injection was given about 48 hours after excision of the ganglion; instillation gave no reliable results in the cat. Cutting the cervical sympathetic nerve below the ganglion in rabbits and in cats brought out no such results. The Meltzers interpreted their findings by the assumption that normally new impulses are originated in the superior cervical ganglion which are of an inhibitory character, an assumption which does not harmonize with Langley's dictum that the post ganglionic nerve fibres are exactly of the same character as those contained in the preganglionic nerve trunk. H. Straub, working in Langley's laboratory, brought forward a few experiments which are supposed to prove that the dilatation of the pupil is a result of the vasodilatation (and



consequently a greater absorption of adrenalin) brought about by the section of the cervical sympathetic. We shall not analyze here the validity of his theoretical discussion. Aside from stating briefly the results of our own experiments, we shall point out that Straub tested only the effect of instillation, using only cats and making the tests immediately after the operation, facts which show clearly that Straub's method of experimentation was entirely inappropriate to test the facts and view brought forward by the Meltzers. As to our experiments there was one important deviation from that of Straub which was essential to the elucidation of the validity of the results under consideration. Straub started soon after the operation to instill every five minutes 3 drops in the conjunctiva of the eye on that side only on which the sympathetic nerve was cut or the ganglion removed, using the other eye as a control. He found that after 90 or 100 minutes the treated eye was maximally dilated. The procedure in our experiments was generally similar to that employed by Straub with the important difference that in our experiments the instillations were made simultaneously in both eyes. The experiments were made upon ten rabbits and nine cats. In about one-third of each species the sympathetic nerve was cut below the ganglion and in two-thirds the ganglion was excised. This difference in the operation made no difference in the results obtained, which were as follows. In the ten rabbits there was an equal dilatation of both pupils in four animals; in the remaining six rabbits the dilatation set in earlier and was greater in the pupil on the intact side. The difference was usually distinct although not great, perhaps only 1 or 2 mm.

In the nine cats the instillations had to be continued for  $1\frac{1}{2}$  to 2 hours. The results agreed with those of the rabbits except that the final dilatation was much less. In two animals the effect was greater on the operated side, while in six the pupil on the intact side remained larger than its fellow.

Our experiments as well as those of Straub show that a frequent prolonged instillation of adrenalin is capable of causing a dilatation even of the normal pupil, a fact demonstrated long ago by Wessely. Our experiments show that the vasodilatation produced by section of the sympathetic nerve or removal of the ganglion have nothing to do with this dilatation of the pupil, and that the prolonged maximal dilatation of the pupil observed by the Meltzers after a single instillation of a few drops or a subcutaneous injection of adrenalin after 24 or 48 hours occurring exclusively after the removal of the superior cervical ganglion, has nothing in common with the dilatation of the pupil following the prolonged saturation of a normal iris with adrenalin.

*Further Studies on Mustard Oil Inflammation.* S. AMBERG, A. S. LOEVENHART AND W. B. McCLURE. From the Otho S. A. Sprague Memorial Institute, Chicago, and the Pharmacological Laboratory of the University of Wisconsin.

An intravenous injection of sodium iodoxy- and iodosobenzoate preceding the instillation of mustard oil in the conjunctival sac of the

rabbit inhibits the inflammatory edema, the degree of inhibition depending on the dosage of these drugs. The sodium iodbenzoate in double the corresponding dosage has no effect, nor did sodium salicylate or a much stronger NaCl solution. The intravenous injection of sodium iodate or periodate also exercised an inhibiting effect on the mustard oil reaction whether the mustard oil was given intracutaneously or instilled in the eye. NaI in double strength was without effect. Similarly the sodium perphthalate inhibits the mustard oil reaction whereas sodiumphthalate does not. Sodium perborate also gave some inhibition while ammonium persulphate showed practically no effect. NaOCl does not strikingly inhibit. In the latter instance the mustard oil reaction of the eye showed a marked tendency to become hemorrhagic, indicating that the character of the inflammatory reaction might be influenced by previous treatment. A number of substances, then, containing active oxygen may exercise an inhibiting influence on the inflammatory reaction caused by mustard oil. The most potent substances were the sodium iodoxy- and iodosobenzoates.

Sodiumiodoxybenzoate was also administered intraperitoneally as well as suspensions of iodosobenzoic acid and benzoylperoxide, all showing an inhibiting effect on the mustard oil reaction of the eye, while 10 per cent NaCl,  $\frac{M}{10}$  sodiumiodbenzoate and gaseous oxygen had no effect. But control experiments where animals received intraperitoneally suspensions of kaolin or blood charcoal or mustard oil also showed an inhibiting effect and that apparently in proportion to the peritoneal irritation produced by these substances. Evidently the results obtained with intraperitoneal injections must be interpreted with the greatest care. Subcutaneous injection of mustard oil may also exercise an inhibiting effect on the reaction obtained or subsequent instillation of mustard oil in the eye. Since several substances containing active oxygen inhibit the inflammatory reaction, possibly by furnishing active oxygen to the tissues, we tried to ascertain whether the procedures likely to interfere with oxidation in the tissues might be capable of intensifying the reaction. Bleeding of animals had no intensifying effect, on the contrary proved somewhat inhibiting. In experiments where animals were exposed to illuminating gas an inhibiting effect was noted more or less proportional to the degree of intoxication. In a series of experiments animals were kept in atmospheres poor in oxygen (7.2-12.4%) under atmospheric pressure from 16 to 84 hours. The results were neither constant nor striking enough to permit any conclusion. Only in experiments where the animals received intravenous injections of NaCN before the application of the mustard oil, evidence of an intensifying influence was obtained. This evidence is the more important as we are justified in the assumption that sodium cyanide actually does interfere with the oxidative processes in the tissues.



## THE EFFECT OF DRUGS ON INFLAMMATION OF THE FROG'S MESENTERY

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### INTRODUCTION

It is stated that inflammatory processes are influenced (1) by those factors which diminish the sensible irritation (analgesics), (2) by drugs which change the calibre and permeability of blood vessels, and (3) by those protoplasmic poisons which paralyse leucocytes.

This enquiry was undertaken in order to ascertain what changes in inflammatory processes may result from the exhibition of such drugs as possess one or more of these effects.

*Method.* I have employed throughout this investigation the method used by Cohnheim (1) in his original experiments. My work has been limited to frogs. These were pithed, and the drug was injected subcutaneously into the thigh. The abdomen was opened and the mesentery exposed to the air and a suitable part was kept under continuous microscopical examination. Further details of the procedure are published in another paper (2).

The drugs which have been used in this work are quinine, cinchonine, ethylhydrocupreine, atophan, mercury perchloride, arsenic, morphine hydrochloride, chloralhydrate, urethane, scopalamine, atropine, magnesium sulphate, antipyrine, colchicum, sodium salicylate, strophanthin, calcium chloride.

The doses used throughout this paper are in milligrammes per gramme of body weight, and only those experiments in which the circulation appeared quite normal throughout the experiment form the basis of this calculation.



## RESULTS OF EXPERIMENTS

1. *Quinine*: The action of this drug has been fully studied by Binz (3) and many other observers. My own results (2) are entirely confirmatory of Binz's work—namely that quinine in a great degree inhibits the migration of leucocytes from the blood stream into the surrounding tissue.

2. *Cinchonine*: This member of the cinchona group was used for comparison with quinine. Cinchonine has no inhibitory effect on the migration of leucocytes even when given in considerable doses. The doses I have tried are from 0.15 to 0.9 mgr., and in each case a severe inflammation set in in one-half to one hour.

3. *Ethylhydrocupreine*: Ethylhydrocupreine is also one of the quinine derivatives. Its sterilising effect on the trypanosoma (4) and pneumococcus (5) infection was studied by Morgenroth and his collaborators. Ethylhydrocupreine is reported to have been used with good results in cases of acute pneumonia (6) and in eye affections (7) caused by the pneumococcus. The experiments were made with ethylhydrocupreine in order to ascertain its effect on the exposed mesenteries. Ethylhydrocupreine had no marked effect though some diminution and postponement of migration was evident. The dose used was 0.012 mgr.

4. *Atophan*: That atophan is an antiphlogistic was pointed out by Starkenstein and Wiechowski (8) on the eyes of rabbits. In my own experiments on frog's mesentery I found that atophan was markedly effective in inhibiting inflammation (2).

5. *Mercury perchloride*: That mercury perchloride constricts the blood vessels was shewn by Heinz (9) on frog's mesentery. My experiments shew that mercury perchloride in a dose of 0.016 mgrm. per frog entirely inhibits the migration of leucocytes. In this dose there was no exudation within 6 to 8 hours.

6. *Arsenic* (Fowler's solution): Liquor arsenicalis is very toxic to cellular life and for this reason it was tested in this research. It was found that 0.0005–0.001 cc. of the solution given hypodermically completely inhibited leucocytic migration. The period of observation was 5 to 6 hours.

7. *Morphine hydrochloride*: Morphine belongs to that group of analgesics which act almost solely through the central nervous system. Januschke (10) demonstrated its antiphlogistic properties on the conjunctiva of rabbits. My experiments upon the frog's mesentery gave a lessening but not total inhibition of the inflammatory process. The doses used were 0.7 to 1.3 mgr.

8. *Chloralhydrate*: Januschke (l.c.) injected chloralhydrate subcutaneously into a rabbit and found that it inhibits the conjunctivitis which mustard oil otherwise causes. In my experiments with frog's mesentery chloralhydrate inhibited distinctly the migration of leucocytes. In doses of 0.4-0.5 mgr. there was no exudation within 4-5 hours.

9. *Urethane*: Urethane is used generally as an hypnotic and as an analgesic. The effect of urethane in my experiments was not so distinct as that of morphine or chloral, though there was some diminution and postponement of exudation. The doses used are 0.5-5.0 mgrs. Even in the doses of 5.0 mgr. there was slight exudation.

10. *Scopolamine* (*Hyoscinæ hydrobromidum*): Though scopolamine has a narcotic action it had in my experiments no effect on the leucocytic migration; it seemed rather to favour inflammation as after the injection the mesentery was found immediately on exposure more extensively injected with migrated cells than in the case of other drugs with the exception perhaps of atropine. The doses used are from 0.05-0.12 mgr. In all cases the inflammation set in at one-half to one hour after the exposure.

11. *Atropine sulphate*: Atropine has in many points the same action as scopolamine. For this reason atropine was tried. The results shewed that atropine has no effect on the migration of leucocytes. The doses used are 0.016 to 0.025 mgr. The inflammation set in from one-half to one hour.

12. *Magnesium sulphate*: That magnesium sulphate has a narcotic action was shewn by Meltzer and Auer (11), and that it acts as an antiphlogistic was demonstrated by Januschke (l. c.) on rabbits' conjunctivæ. But in my experiments magnesium sulphate shewed with the doses employed (0.8 to 1.85 mgrs.) no notable effect on the inflammation in the frog's mesentery. The inflammation set in from one to one and one-half hours.

13. *Antipyrine*: Antipyrine is an antipyretic and an analgesic and Januschke (l. c.) has proved its antiphlogistic action on the conjunctivae of rabbits. In my experiments with frog's mesentery antipyrine strongly inhibited the migration of leucocytes. The doses used are 0.6 to 1.0 mgr. There was no exudation within 5 to 6 hours.

14. *Colchicum*: Colchicum is used as a specific remedy for acute gout, and may have some effect on inflammation. But in my experiments colchicin had no effect in inhibiting the migration of leucocytes in the exposed frog's mesentery. In doses of 0.014 to 0.04 cc. of saturated solution of colchicin the inflammation set in as usual from one to one and one-half hours after exposure.

15. *Sodium salicylate*: Binz (12) shewed that sodium salicylate paralyzes the active motion of leucocytes. Salicylic acid and its salts are known as a specific remedy in acute rheumatism, and salicylates exert a definite antipyretic and analgesic action and perhaps act as antiphlogistics. Januschke (l. c.) confirmed the antiphlogistic action on rabbits' conjunctivae. In view of these facts I tried sodium salicylate but in my experiments sodium salicylate (in doses of 0.2 to 0.5 mgr.) had absolutely no effect upon leucocytic migration. The inflammation set in in one to two hours.

16. *Strophanthin*: Strophanthin and digitalis are used in the treatment of pneumonia. To ascertain if these drugs possess any inhibitory effect on inflammation I made several experiments with strophanthin, but it proved useless as an inhibitor of leucocytic exudation in doses of 0.00045 to 0.0005 mgr. The inflammation set in in one to one and one-half hours.

17. *Calcium chloride*: Wright (13) pointed out that urticaria and serum exanthems could be treated with good results by the administration of calcium chloride. Chiari and Januschke (14) have found that the inflammation of the pleura and of the pericardium caused by iodine or thiosinamine in dogs and guinea pigs and the conjunctivitis caused by mustard oil in rabbits can be inhibited by the hypodermic injection of calcium chloride. Luithlen (15) has tried the effect of hypodermic injections of



calcium chloride on inflammation caused by croton oil on the skin of the cat, and found that this salt inhibits the inflammation. This antiphlogistic action of calcium chloride was subsequently confirmed by many authors. The results of my experiments shew that calcium chloride also inhibits the migration of leucocytes in inflammation of frog's mesentery. In doses of 0.7 to 1.3 mgrs. there was no exudation within 5 to 7 hours.

The following experiments were carried out in view of the possibility of the synergetic action between those antiphlogistics. The combinations tried were:

(1) Morphine (0.04 to 0.23 mgr.) and scopolamine (0.0003 mgr.)

(2) Quinine and atophan. See reference 2.

(3) Quinine (0.008 to 0.032 mgr.) and urethane (0.015 to 0.024 mgr.).

(4) Quinine (0.036 to 0.044 mgr.) and ethylhydrocupreine (0.0017 to 0.0021 mgr.).

(5) Ethylhydrocupreine (0.0015 to 0.0016 mgr.) and sodium salicylate (0.15 to 0.16 mgr.).

Among these combinations that of quinine and atophan was the only one which gave positive results, at least in the dosage I have tried. The results of quinine and atophan are published in a separate paper (2).

#### SUMMARY.

1. Drugs producing marked inhibition of leucocytic migration: quinine, atophan, mercury perchloride, arsenic, antipyrine, morphine, chloral, calcium chloride.

2. Drugs producing only slight inhibition: ethylhydrocupreine, urethane.

3. Drugs producing no inhibition: Cinchonine, scopolamine, atropine, strophanthin, colchicum, sodium salicylate, magnesium sulphate.

4. Certain drugs, as quinine and atophan, when combined show a synergetic action in inhibiting the migration of leucocytes.



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## THE SEGMENTAL ACTION OF STRYCHNINE

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In their study of the site of strychnine action, McGuigan and Becht (1) showed conclusively, that, under the conditions of their experiments, the action is decidedly segmental. Since this work was carried out on anesthetized animals the disturbing action of the ether, which may also be somewhat segmental in action, was not sufficiently considered. The present work considers the action on normal unanesthetized animals. It is sufficient to report a few typical experiments only.

In the work quoted the physiological fact was emphasized that the normal spread of motor impulse and action is centrifugal. Consequently when strychnine is applied to the cervical region of the cord the tetanus spreads more readily caudalward than cephalad. A due consideration of this fact practically nullifies the conclusion drawn by Houghton and Muirhead (2), Baglioni (3) et al.,<sup>1</sup> that the action of strychnine is more on the sensory than on the motor parts of the cord. Apropos of this work we wish to cite an experiment with frogs in which the strychnine is generally distributed, and which gives the identical result which they thought was obtained because of the local action of the alkaloid.

As is well known, Houghton and Muirhead destroyed the circulation of a frog to prevent the spread of the administered

<sup>1</sup> Barenne appropriately (*loc. cit.*) points out the differences between the methods of Houghton and Muirhead, and of Baglioni who arrived at the same conclusion as the former but by a different method. Baglioni thought he eliminated the sensory neurons by the local application of phenol. Because of the same conclusion, the method of Houghton and Muirhead has been sometimes incorrectly referred to as that of Baglioni.

drug. Then, they exposed the cord high up in the cervical region and applied strychnine to it in that region only. After a time stimulation of the front leg evoked a general tetanus while the same stimulus applied to the hind leg caused a normal reflex movement of the hind leg only. From this they concluded that strychnine can cause tetanus in areas where it is not present if the impulse passes through a poisoned sensory area to an unpoisoned motor area, but if the impulse passes through an unpoisoned sensory to a poisoned motor region no tetanus is evoked. They concluded, therefore, that the sensory cells only need be poisoned to produce tetanus. In their work on dogs, Becht and McGuigan (*loc. cit.*) have called attention to the erroneousness of such conclusions and the following easily performed frog experiment supports their opinion:

*Experiment I.* If 0.1 to 0.2 mgm. strychnine sulphate per 20 grams of body weight be injected into the anterior lymph sac of a frog tetanus will develop in the course of some minutes. When tetanus has developed the animal may be decapitated. After a brief period of shock tetanus again develops and the strychnine is spread through the body. A certain minimal stimulus can easily be found, which when applied to the front leg will cause a general tetanus, but, when applied to the hind leg will produce movement of the hind legs only. We have repeatedly confirmed this fact. Yet this is the same result Houghton and Muirhead (*loc. cit.*) obtained and which they attributed to the local action of strychnine in the cervical region. It is obviously the simple fundamental physiological reaction which we have stated above, changed only in degree by strychnine.

*Experiment II.* We have confirmed the facts stated above regarding the spread of the motor action in dogs under ether and strychnine as follows: A dog 14 kilos, was etherized and the cord exposed in the cervical, dorsal and lumbar regions. 0.5 mgm. of strychnine sulphate was injected into the cervical and lumbar parts of the exposed cord. In a few minutes the muscles supplied by these areas were very hypersensitive and responded readily to stimulation. Stimulation of the dorsal exposure with a minimal interrupted current from a secondary coil always caused a contraction of the hind legs more easily than the front. With a current of two volts passing through an induction coil, there was a contraction of the hind legs when the secondary coil

was removed 10 cm. from the primary and of the front legs only after the coil had been advanced to within 3 cm. of the primary. Evidently a much stronger stimulus is necessary to elicit a motor response cephalad than caudalward.

*Experiment IV.* Action and spread in the unanesthetised mammal. Dog 14 kilos.

- 12.38 1 mgm. of strychnine injected into the cord in lumbar region.
- 12.43 Tetanus in the hind legs—front not involved.
- 12.50 Tone of the front legs increased.
- 12.50 Animal can walk but the hind legs are very spastic. The gait is ambling and in walking the legs are held stiff and raised but slightly from the floor. The animal can step over an obstacle 20 cm. high with the front legs but not with the hind legs.
- 12.55 Sacro-iliac joints seem locked—so that the hind legs are tetanic and move together in a hopping gait; a striking illustration of Sherrington's (4) view of the action of strychnine.
- 12.56 General tetanus with the head but little involved. Time for the strychnine to travel from the lumbar region until the neck and head are involved about 20 minutes.
- 12.58 On recovery from this spasm the animal was given 4 grams of urethane. Three days after the animal was in good health.

*Experiment V.* Dog 7 kilos. No anesthetic at commencement.

- 10.51 0.5 mgm. strychnine sulphate in 0.5 cc. water injected into region of the fourth ventricle.
- 10.52 Tetanus in head region and front legs.
- 10.53 Terrific scratching of the head with the hind legs, similar to the movements after morphine (5). Not sensitive posterior to the saddle area. Respiration greatly stimulated.
- 10.55 Spasm in front spreading gradually backward.
- 10.56 General strong spasm—with hind legs but little tetanic—can still scratch the head with them, but they are distinctly increased in tone. Time of the spread backward from the fourth ventricle to the hind leg area—about five minutes. This is decidedly shorter than the spread in the opposite direction.
- 10.58 Ether given to relax the spasm.
- 11.02 4 cc. of cerebrospinal fluid withdrawn from the lumbar region and 1 cc. of strychnine solution—1 mgm.—injected.<sup>2</sup>

<sup>2</sup> It is usually hard to withdraw cerebrospinal fluid from a dog in this region. The failure to get fluid, is not proof that the needle is not in the proper place.



- 11.04 Strong spasms in both front and hind legs.
- 11.05 Ether administered.
- 11.06 On letting the animal out of the ether the hind legs go into tetanus while the front legs are in swimming movements. This suggests that the front part of the nervous system is more influenced by ether and remains longer affected than the lumbar region.

The results here are definite and easily obtainable. As in the case of the frog experiments they show that the spread of strychnine is more rapid caudalward than cephalad and that the motor response to stimulation spreads more readily caudalward than cephalad, so that when the hind legs are stimulated it is easy to obtain a response of the hind leg only. While this fact in itself will not permit of any definite conclusion regarding the site of the action of strychnine—since it is the normal response of the animal exaggerated by the presence of strychnine—it is very suggestive of the motor action of strychnine. The same conclusion can be drawn from the action of strychnine on anaesthetized animals.

The action of strychnine in normal unanesthetized as in anaesthetized animals is the same, except that the rate of spread of the action appears greater in the normal state. This is probably due to the greater movements of the animal. The work supports the previous opinion based on the work of McGuigan and Becht that the motor elements of the cord are involved directly in the action of strychnine.

Sherrington (6) cut the dorsal sensory roots between the ganglion and the cord and found that the action of strychnine was still effective after six weeks when all the parts of the cut neuron must have undergone Wallerian degeneration. The action would seem, therefore, to be on the motor neuron. The objection, however, has been raised that degeneration would stop at the periphery or end of the cut neuron and would not cause the degeneration of the intercalated cells within the cord. While we believe that such conclusions are improbable, their possibility must be admitted, but in view of the evidence we have already presented against the theory that the sensory cells only are

involved in strychnine action, we feel that assumptions in favor of the sensory and against motor involvement can no longer be admitted. The evidence is decidedly in favor of a simultaneous motor action. Barenne (7) also advances strong evidence in favor of the direct motor involvement.

By the use of cocaine and strychnine we have obtained some evidence that strychnine acts on the motor neurone. The cocaine has the same effect as cutting the dorsal roots without the actual solution of continuity, although, as we will show, caution is needed in drawing conclusions from cocaine work.

*Experiment I.* If a 0.1 per cent solution of cocaine be injected into the lumbar cord of a normal animal, a loss of feeling below the injection will soon be manifest. If now a tetanic dose of strychnine be given in the same region tetanus of the hind legs may be elicited readily by direct stimulation or by stimulation of the cord above the cocainized area. Sensation is distinctly lost below the cocaine.

*Experiment II.* Since the same objections may be raised in this case as in the work of Sherrington we have modified the conditions of the experiment to avoid objection so far as possible. We have, therefore, in an anaesthetized animal laid bare the cord in the lumbar region and injected cocaine into it until stimulation below the cocainized part caused no response forward while stimulation above the block caused general response. Then strychnine was injected and found still to be effective, and caused only local action in the unaesthetized part; stimulation below or over the cocainized-strychninized area caused no movements in the front part of the animal.

*Experiment III.* Since the objection again may be raised that cocaine and strychnine are antagonistic in their action on the cord and that the strychnine stimulated the parts paralysed by cocaine, we have reversed the order of administration and given the strychnine until tetanus was fully developed and the slightest touch of the hind leg caused tetanus; then cocaine was administered as above. After a time strong stimulation of the leg evoked no tetanus. Stimulation over the poisoned areas caused local tetanus only with no spread to the front—showing an action on the sensory neurons by the cocaine, while stimulation of the thoracic cord caused a tetanus of the hind legs. Also in some cases we have found stimulation of the motor areas effective, but not often, and in these cases positive results soon become negative due to the action on all the elements of the cord.

*Experiment IV.* Since the fundamental or primary segmental reflex is difficult to abolish entirely with cocaine without at the same time destroying conduction in the motor element, in the above experiments we may not have entirely eliminated the deep seated intercalated neurons within the cord while pain and response to superficial stimuli were absent. We have therefore isolated a branch of the sciatic and waited after the injection of cocaine until the abolition of the sciatic reflex. Then strychnine was injected and stimulation of the cord in the dorsal region gave decided tetanus in the hind legs. However, the sciatic reflex had returned, and it is clearly apparent that cocaine and strychnine are antagonistic when administered in this way and in this location. The whole of the work on cocaine and strychnine must remain therefore, more a matter of opinion than a clearly demonstrable action. Strychnine, however, still acts vigorously when the long reflex arcs are paralysed and is decidedly segmental in action, and it would seem that only the most primitive reflex arcs are necessary. It may be argued that since strychnine is antagonistic to cocaine it acts on the same point. We admit this, but since cocaine acts on both sensory and motor neurons its action can not be used to definitely locate the site of strychnine action on the sensory neurons only. We think, however, that since cocaine acts more readily on the sensory cells and fibers than on the motor, the quick recovery after an injection of strychnine indicates at least some action by the strychnine on the motor element. To illustrate the antagonistic action of cocaine and strychnine in the cord we will give the result of one experiment.

. *Dog, 20 kilos*

- 9.00 Ether. Cord in dorsal and lumbar regions exposed and a branch of the sciatic exposed, ligated and cut with the central end prepared for stimulation. The sciatic responsive at 20, dorsal responsive downward at 13 and up at 8. (The figures refer to the distance in centimeters of the secondary coil from the primary.)
- 9.38 0.5 cc. cocaine 0.1 per cent injected subdurally in lumbar region.
- 9.43 Dose of cocaine repeated.
- 9.44 Sciatic reflex dead at 0. Dorsal down strong at 6.
- 9.45 2 cc. 0.1 per cent strychnine sulphate in lumbar region.
- 9.46 Sciatic reflex at 20, dorsal at 7.5. Lumbar does not go up at 0, but causes tetanus in hind legs.



- 9.50 Tetanus easily elicited by tapping hind legs and becoming spontaneous.
- 9.51 Sciatic responsive at 21, dorsal down at 20.
- 9.52 1.5 cc. cocaine 0.1 per cent.
- 9.59 Sciatic responds at 20, dorsal at 7.
- 10.00 1.75 cc. cocaine solution in lumbar cord.
- 10.03 Sciatic stimulation negative at 0, dorsal positive at 10.
- 10.08 2 cc. strychnine 0.1 per cent in lumbar cord.
- 10.15 Sciatic positive at 16, dorsal at 15.
- 10.37 Sciatic and dorsal both positive at 25. No transmission upward from this stimulation in either region.

Cocaine again paralysed the reflexes.

While cocaine and strychnine are antagonistic on the cord strychnine will not antagonize the action of cocaine on respiration.

To test whether impulses passing through a strychnized area are modified in their effects beyond those areas, the following experiments were performed which indicate that an impulse is but slightly, if at all, modified in passing through a strychninized area.

*A dog, weight 4 kilos*

- 10.15 Ether.
- 10.45 Cord exposed in lumbar and upper dorsal regions, and the motor areas of the brain laid bare. The object of this procedure is to get an area which may be strychninized and through which we may send impulses in either direction.
- 10.53 Stimulation of the motor areas causes movement of hind and front legs at 7.5 cm.
- 10.55 Stimulation of the lumbar region gives response down at 20, up at 3. Dorsal region, down at 25, up at 14. This region of stimulation is close to the front legs.
- 11.00 0.4 cc. of a 0.5 per cent strychnine sulphate injected subdurally into the upper dorsal region. Almost immediately the reflexes over this area become much more easily elicited.
- 11.05 Front leg from motor area responds at 8.5. Hind leg only at 3. Lumbar stimulation goes down at 25. Dorsal down at 25 and up at 20.<sup>3</sup>

<sup>3</sup> The dorsal point of stimulus is very close to the front leg area.



- 11.10 The strychnine seems to have spread downward. The reflexes in the hind legs are markedly increased on local stimulation—no spread forward, although the animal is lying on a level surface on his belly.
- 11.15 Motor areas of both legs respond at 9.5.
- 11.20 Tail very sensitive and reacts to slight external stimulation. It seems that the motor cells to the hind legs and tail—some of them—may arise near to the point where the strychnine was applied, else the strychnine is traveling down the cord.
- 11.25 Front legs react from stimulation of the motor areas at 9.5. Hind legs at 8.0.
- 11.30 0.3 cc. more strychnine 0.5 per cent in dorsal cord.
- 11.35 Light stimulation anywhere behind shoulders cause reflex contractions. No response if stimulation is made anterior to the shoulders.
- 11.36 Front legs respond from stimulation of the motor areas at 10. Hind legs at 9. Note this result. The front legs at this time have gone into spontaneous tetanus, stimulation of motor areas immediately preceding the tetanus, while it usually gives a more vigorous response to the stimulus, yet the stimulus required to elicit the response is just as strong as before the administration of the strychnine.
- 11.36 After strong tetanic spasm for some time the front legs are quiescent.
- 11.44 Stimulation of the lumbar region at 11 causes strong tetanus in the hind legs—no action in front. This is not because the front legs are paralysed from the previous spasm, because we get a good response of the front legs from the motor areas at 9.5 and tetanus at 8.
- 11.50 Hind legs responsive from dorsal region at 13 and go into tetanus at 7.5. All stimulations of the motor areas so far were made through the dura.
- 11.55 Dura removed.
- 12.00 Front leg responds from motor area at 10; hind leg can not be made to move.
- 12.04 0.5 cc. more strychnine in dorsal cord.
- 12.08 Lumbar region stimulation at 9.5 gives tetanus of the hind legs only. This does not spread forward although all areas posterior to the shoulder are very sensitive. Motor areas send the front legs into tetanus at 9, and this does not go to the

hind legs. There seems to be a block between the shoulder and hind legs areas. A region of lower sensitivity in close proximity to one of higher sensitivity.

- 12.14 0.5 cc. strychnine in lumbar region.
- 12.15 Local continuous spasms in the hind legs, which show no tendency to go forward.
- 12.18 0.5 cc. strychnine 0.5 per cent in region of the fourth ventricle—soon causes spasms of the head and neck, becoming general.
- 12.21 1.5 cc. of 0.1 per cent cocaine in fourth ventricle.
- 12.22 Respiration stopped.
- 12.23 Strychnine slowly injected fails to cause stimulation of respiration. This is the usual result. We can not find that strychnine and cocaine are antagonistic to the respiration although they are in the lower portions of the cord.

The modification of impulses by passage through strychninized areas.

*Experiment II. Dog, 4 kilos.*

- 11.30 Ether, and motor areas, dorsal cord and lumbar cord exposed.
- 12.00 Front leg area responds at 8 cm. Hind leg at 7.
- 12.18 0.5 cc. 0.1 per cent strychnine in upper dorsal region.
- 12.20 Higher sensitivity of regions over the areas but no change on the leg responses.
- 12.21 0.5 cc. more strychnine in the dorsal region.
- 12.22 Tetanus locally on stimulation of the dorsal segment.
- 12.23 Hind leg responds at 6 cm. Questionable at 7. There seems to be some depression rather than stimulation. The ether has been kept constant. No response can be elicited in front from stimulation of the lumbar region although the stimulus is sufficient to cause tetanus of the hind legs.

A number of other tests were made but in no case did the motor impulse from stimulation of the motor areas become more effective or more sensitive in passing through the strychninized areas. This also holds for the reverse direction. Impulses passing from the lumbar region to the anterior part of the animal are not more effective or more sensitive by passage through a strychninized area.

- 1.15 The animal was given 0.5 cc. cocaine hydrochloride 0.1 per cent into the fourth ventricle. Respiration stopped immediately. The animal was then given 1 cc. strychnine 0.1 per cent into the same region. Strong tetanus was soon developed but respiration did not return. This is the usual result.

That the impulse passing through the strychninized area is not modified agrees with the well known fact that the posterior root ganglia are but little concerned in a general strychnine action and that when they are painted with strychnine no tetanus develops unless the drug has spread throughout the body (Barenne). Since the ganglion is not involved especially in the action and stimulation central to the ganglion produces tetanus, the point of action has been referred to the synapses between the posterior root and the motor cell—a very elusive site.

Poulsion (8) has shown that if a tetanized frog be painted with cocaine, or if one foot be dipped in a cocaine solution of 5 per cent, stimulation of the cocainized area will fail to evoke tetanus, while it can still be shown that the motor cells or neurons are intact. We have repeated this experiment and can easily verify the statements. A few words of explanation in this regard are necessary since this has become a routine laboratory experiment, and a wrong conception of the action of strychnine may be drawn from it. Any co-ordinated movement either in the normal or in the strychninized animal requires a stimulus from the sensory side to initiate it. Without such a stimulus, such co-ordinated movement is improbable. In other words, both sensory and motor portions of the arc are necessary. If, therefore, we anesthetize or cut the sensory neurons in any way either by cocaine, ether, chlorotone, or by complete section, no such action can take place. None should be expected. The experiment cited throws no light on the point of action of strychnine but merely shows that the sensory elements of the arc are involved in the response of the strychninized animal as in the normal animal. Strychnine painted on the skin soon exerts its general action and there is no reason to suppose that cocaine will act differently. The fact that light stimulation of the skin fails to cause tetanus while stimulation of the joints does, simply means that the deep



sensory fibres have not been reached by the cocaine. A prolonged action or direct application to the sciatic will abolish the results of all stimulation deep or superficial.<sup>4</sup>

#### CONCLUSIONS

1. The normal spread of motor impulses is caudalward and therefore a stimulus which produces a general movement of the body when applied to the front leg, may cause only a local reflex when applied to the hind leg. This relation is not changed by strychnine.

2. The direction of spread of motor impulses is the same in the normal animal as in the anaesthetized animal but the rate of this spread is apparently greater in the normal than in the anaesthetized animal.

3. By the use of cocaine and strychnine, evidence is gained which supports the view that some of the strychnine action is due to a direct action on the motor neuron.

4. Strychnine injected into the lumbar cord of an unanaesthetized animal gives a vivid illustration of deranged reciprocal innervation thus confirming the theory of Sherrington concerning strychnine action.

5. Neither sensory nor motor impulses are modified in their end effect by passage through a strychninized segment of the cord, unless the effector neuron arises from the poisoned segment.

6. Strychnine spreads downward in the cord more quickly than upward. This condition is also suggestive of motor involvement by the strychnine.

<sup>4</sup>The action of strychnine and cocaine requires investigation. Dr. F. C. Becht tells us that if a frog be dipped in 1 per cent cocaine solution, and again dipped after a few minutes, it will react more quickly to strychnine than a normal control. There are other changes in the cocainised animal that enables one to differentiate frogs in cocaine-strychnine tetanus, from the simple strychnine tetanus.



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## ON THE PHARMACOLOGY OF THE URETER

### I. ACTION OF EPINEPHRIN, ERGOTOXIN AND OF NICOTIN

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#### INTRODUCTORY

Although the physiology of the ureters has been pretty thoroughly investigated by Engelmann (1) and Protopopoff (2) in their classical contributions on the subject, and by Sokoloff and Luchsinger (3), Lewin and Goldschmidt (4), Fagge (5), Henderson (6), Sollmann (7), and others, the pharmacology of those important organs is practically unknown. Almost all observations on the influences of drugs upon the ureter to be found in the literature have been made incidentally in connection with other strictly physiological problems. Even Lucas (8) who has so far made the most important contribution on the subject and to whom the credit belongs of first noting the tonic action of epinephrin on the ureter, did so in connection with his valuable contribution on ureteral pressure, and freely confesses his uncertainty in regard to his other pharmacological observations. Quoting from his paper (p. 272), we read: "In the experiments with the excised ureter by the method described above, nicotin, atropin, muscarin, and physostigmin gave only negative results (but I feel confident that these drugs exert definite influences and that they can be demonstrated graphically by improvement of the technique)." A systematic inquiry into the action of pharmacological agents on the ureters is therefore very desirable and has been accordingly undertaken by the author.

## METHODS

The behavior of the ureters can be studied in three ways: firstly, by simple observation of their movements *in situ*; secondly, by cannulating them and recording their contractions with suitable apparatus; and thirdly, by studying surviving excised ureters. In the present investigation the most convenient and effective method of studying the action of drugs on the ureter was found to be by the last-named method. Two previous observers, Lina Stern (9) and Lucas (8) have made studies of the excised organs. The work of the latter has already been referred to. The pharmacological data of Lina Stern are not reliable, as that author made observations on excised ureters suspended in normal saline solution. It is well known that isolated organs or tissues, as for instance, the heart, the intestines, or the uterus rapidly die in a solution of NaCl, a proper admixture of other salts, such as in Ringer's or better still Locke's solution, being essential to sustain their life. It is therefore not surprising that her experiments gave negative results.

In the present research a different method was employed from that of Stern and Lucas. These authors employed pieces of the whole ureter from small animals—cats, dogs, rats, rabbits, and guinea pigs. Now it is well known that the musculature of the ureters is strongly developed, and consists of two principal layers, an inner longitudinal and an outer thicker circular one. Some longitudinal strands outside the circular are also described by some histologists. A simple inspection of a live ureter *in situ* is sufficient to demonstrate that, just as in the case of the intestines, it is the latter or circular layer of muscle which is chiefly concerned in producing the peristaltic contractions and maintaining the tonus of the organ. It is evident that through the study of suspension preparations of portions of the whole ureter, the previous observers recorded for the most part the movements of the longitudinal muscular layer. In the present investigation, the author endeavored to study the behavior of the longitudinal and of the circular muscles as much as possible separately. The first was done by suspending longi-

tudinal strips of a slit ureter. The latter was studied by the use of *ureteral rings*, a method that so far as is known, had never been previously employed. For this purpose the ureter of larger animals, pigs or oxen, were required, and that of the pig was found to be the most suitable, and convenient. It was soon learned that the ring method is by far the most useful for studying the influence of drugs on the ureter. It is a method, which is furthermore admirably applicable to the study of the *human* ureter, for a small piece of ureter, a fraction of a centimeter in length, can be generally procured with little difficulty from patients undergoing a nephrectomy operation.

#### PHYSIOLOGICAL

When a ring of pig's ureter 2 to 3 mm. thick is suspended in a glass chamber full of Locke's solution at 37°C. or body temperature, through which a constant stream of oxygen is kept

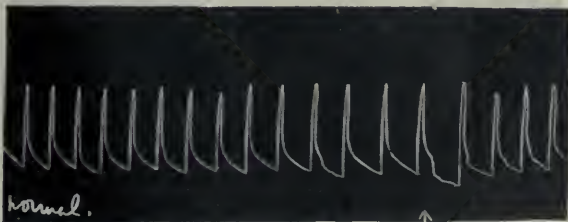


FIG. 1. Ring of pig's ureter six hours after death. Experiment, January 12, 1916. Showing normal contractions in normal Locke's solution. Contraction = up-stroke; four beats per minute.

bubbling, it is at first quiescent. After a period of time, however, varying from ten minutes to an hour, it begins to contract spontaneously at regular intervals, and the curve inscribed by the contractions with a light lever on a revolving drum, looks not unlike a tracing made by a suspension preparation of a frog's heart (fig. 1). Very much the same though not as uniformly satisfying results are obtained by the use of plain Ringer's solution. In normal NaCl solution, however, the contractions, do not develop, and if started up, are feeble and soon



die off. In this respect the ureter behaves like other smooth muscle tissues, which have been shown by Hedon and Fleig (10), Manevich (11), and others, to require a suitable balancing of K, Na, and Ca ions for the preservation of their vitality. After some experimentation it was found that the addition of small quantities of urea (0.2 to 0.4 per cent) improved the ureteral contractions, and that the optimum medium for the study of the above-described phenomena was obtained by mixing the Locke solution with fresh urine in proportion of about 10 to 1 (fig. 2). The addition of a little urea or urine to a Locke solution was seen often to revive the contractions of even a quiescent or non-contracting ureteral preparation. Such a medium it will be noted closely approximates to the conditions in the living body.



FIG. 2. Ring of pig's ureter twenty-four hours after excision. Experiment January 13, 1916. Showing normal contractions in Locke, and the improvement of the same by addition of urea 0.1 per cent. Contraction = up-stroke; normal four beats per minute; after urea seven beats per minute.

Exactly the same results are obtained with the human ureter from a case of nephrectomy (fig. 3).

In regard to oxygenation our experiences agreed with those of Lina Stern and were contrary to those of Lucas. Lack of oxygen was found to diminish the activity of the ureter, while efficient oxygenation of the fluid medium favored it.

The effect of temperature was interesting and important. The optimum temperature was found to be about 36° or 37°C. Variations in temperature between 34° and 38°C., produced but slight change in the rate and force of the contractions. Higher and lower temperatures, however, markedly decreased the move-



FIG. 3. Human ureter; one ring; four hours after nephrectomy for hydro-nephrosis on December 15, 1915; showing spontaneous contractions in normal Locke. Contraction = up-stroke, seven beats per minute.

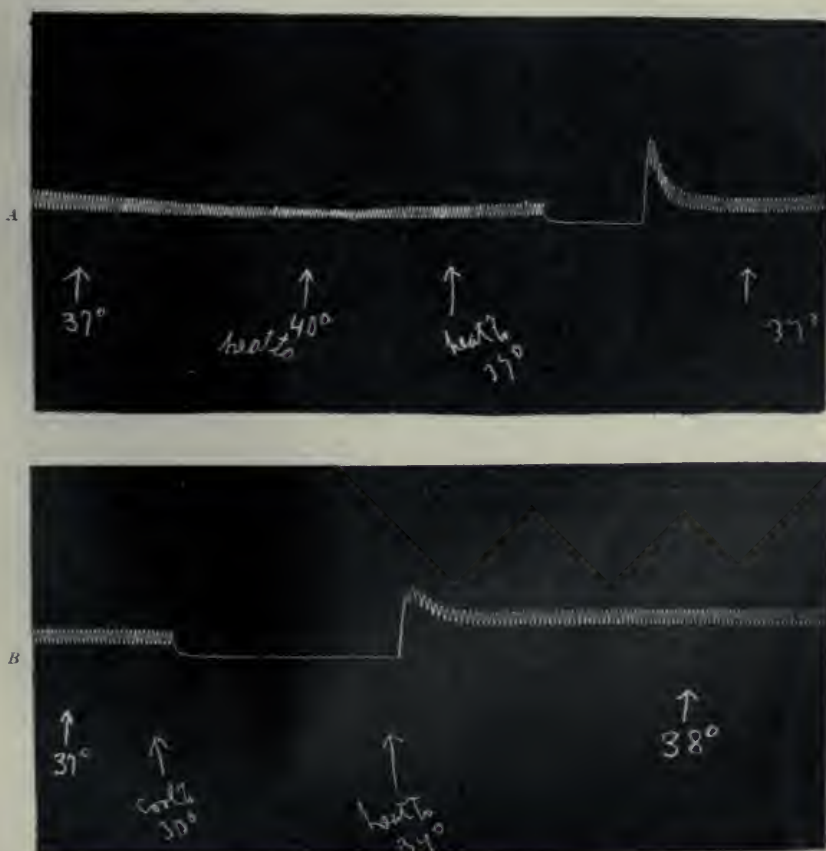


FIG. 4. Experiment December 21, 1915. Ring of pig's ureter three hours after death. A, Showing effect of heating; B, showing effect of cooling. Speed of drum, about six beats per minute at 37°C.

ments and finally paralyzed them, the upper and lower limits being  $41^{\circ}$  and  $32^{\circ}\text{C}.$ , respectively. Restoring the temperature to normal revived the contractions (fig. 4).

The above is the behavior of the ring preparation or circular muscles of a ureter, under normal physiological conditions. The behavior of longitudinal strips of the ureter was somewhat different; inasmuch as the longitudinal muscles normally exhibit very little or no rhythmical movements or contractions. In respect to their reactions to drugs, however, both longitudinal and circular muscle preparations qualitatively showed the same changes. For most purposes therefore the ring preparations were by far the most instructive to study, as they indicated the effect of drugs on peristalsis and tonicity.

#### ACTION OF EPINEPHRIN

The action of epinephrin (adrenalin) on the ureter is of great interest as throwing light on the still disputed question of its innervation. As is well known through the work of Langley (12) and Elliott (13), epinephrin is our principal sympathetic poison or drug, attacking all sympathetic or dorsal lumbar autonomic (Langley) nerve-endings or as some prefer to express it myoneuronal junctions—whether pressor or depressor, accelerator or inhibitor, constrictor or dilator, wherever they may be—and stimulating them to greater activity, just as an electric current would do. The response of a tissue to epinephrin, is therefore generally regarded as an indication of its innervation by the sympathetic.

When a minute quantity of epinephrin (one drop of a 1: 10,000 solution) is introduced into a chamber filled with 50 cc. of Locke's solution and containing an ureteral ring preparation, a striking effect is soon noticed. The ureter begins to contract more frequently and more vigorously, and its tonus is distinctly increased. Such a small dose of epinephrin will start powerful contractions in an otherwise quiescent ureter. It is interesting to note that the vitality of an excised ureter, like that of an excised artery (14) if kept on ice in Locke's solution, can be preserved for quite a

long time. I have seen ureters two and three days after excision respond to epinephrin, by beginning to contract rhythmically (fig. 5).

Larger doses of epinephrin exert a correspondingly greater influence on the ureteral ring. The contractions become very

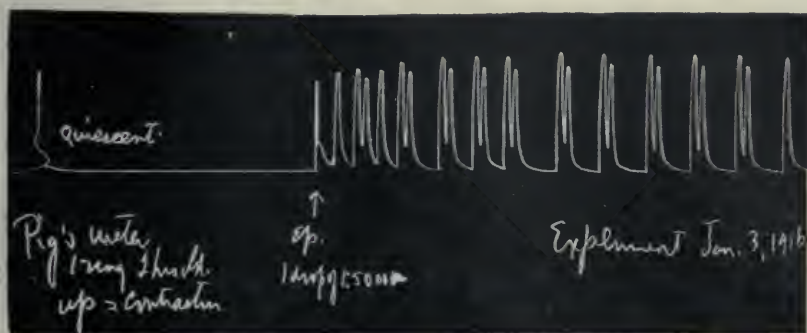


FIG. 5. Quiescent ureteral ring, from pig stimulated to powerful contractions by a minute dose of epinephrin, 1 drop of 1:5000 solution in 25 cc. of Locke.



FIG. 6. Pig's ureteral ring, twenty-two hours after excision; showing stimulating effect of 1 mgm. epinephrin in 30 cc. Locke; wearing off of effect one-half hour later; and powerful tonic spasm from 5 mgm. of epinephrin.

rapid, and at the same time very short, owing to the greatly increased tonicity. Indeed the ureter finally passes into a condition of tonic contracture or tetanus (fig. 6). This lasts for an hour or more and gradually wears off, as the drug is oxidized, until the contractions become normal again.



A ring of human ureter reacts to epinephrin in exactly the same way.

It is interesting to note furthermore, that a similar effect is produced by epinephrin on a longitudinal ureteral strip. Such a preparation, while ordinarily quiescent (fig. 7), may be excited to rhythmical contractions by the drug.

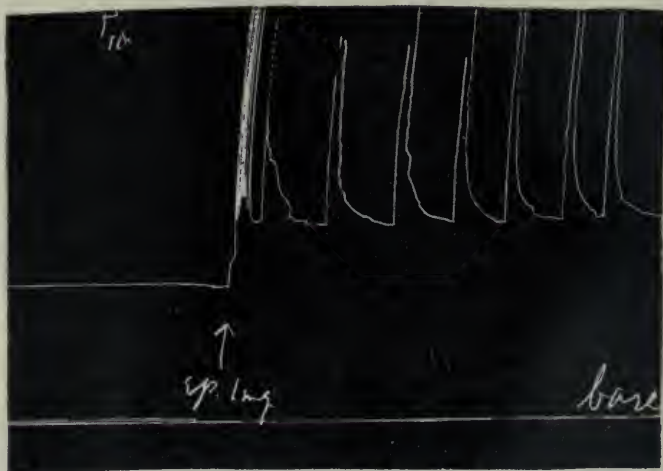


FIG. 7. Longitudinal strip of pig's ureter, caused to contract by 1 mgm. of epinephrin in urine-Locke.

The action of epinephrin just described argues strongly for the presence of sympathetic terminals in the ureteral walls. This is further corroborated by the reaction of the ureter to ergotoxin.

#### THE ACTION OF ERGOTOXIN

The chemical and physiological properties of the principal active constituent of ergot have been carefully investigated by Barger and Dale (15), (16). Its physiological effects, as a result of their work, fall into two groups.

a. A stimulant effect on plain-muscular organs, prominent among which are contractions of the arteries, the uterus, and the sphincter of the iris.

b. A specific paralysis of the *motor* elements in the structures associated with sympathetic innervation, which adrenalin stimulates; the inhibitor elements retaining their normal function, as do also both motor and inhibitor autonomic nerve supplies of cranial and sacral root origin.

As a result of this peculiar selective action, the effect of epinephrin on those organs which are supplied with both motor and inhibitor sympathetic terminals, as for instance, the arteries of carnivora, and in which ordinarily epinephrin produces a motor effect, is *reversed*. Thus an animal which is first injected with ergotoxin, will respond to a subsequent injection of epinephrin with a fall instead of the ordinary rise in blood-pressure, because the motor-terminals having been paralyzed by the ergo-



FIG. 8. Experiment January 12, 1916. Pig's ureter; one ring in urine-Locke (1:10). Showing effect of ergotoxin 5 mgm. and reversal of epinephrin action following same. Contraction = up stroke.

toxin, the suprarenal principle can act only on the inhibitor terminals which are still intact.

The author of the present paper has shown in a previous communication (17), that this "reversal" phenomenon can be demonstrated not only by blood-pressure experiments in the whole animal, but also, very conveniently, by the use of arterial rings *in vitro*. It was therefore interesting to try the action of ergotoxin on ureteral rings.

When a ureteral ring preparation is treated with a small dose, 1 to 2 mgm. of ergotoxin in 50 cc. of Locke, there is soon noticed an increase in the tonus and in the rate of contraction—a primary stage of stimulation. A little later the contractions become slower and the tonus slightly relaxes, specially if a larger dose of the drug is used. If now, the ergotoxin is followed by

a dose of epinephrin, it is interesting to find that the motor effect of epinephrin is inhibited, and indeed, a slight relaxing effect is noted. In other words, the ureter exhibits a reversal of epinephrin action after ergotoxin, in exactly the same way as an artery would do (fig. 8). This fact is of scientific interest, being, as far as is known, the first demonstration of a reversal of epinephrin action in case of a peristaltic organ. The intestine, which is the peristaltic organ par excellence, does not lend itself to the experiment, as according to Dale its sympathetic supply is exclusively of an inhibitory character.

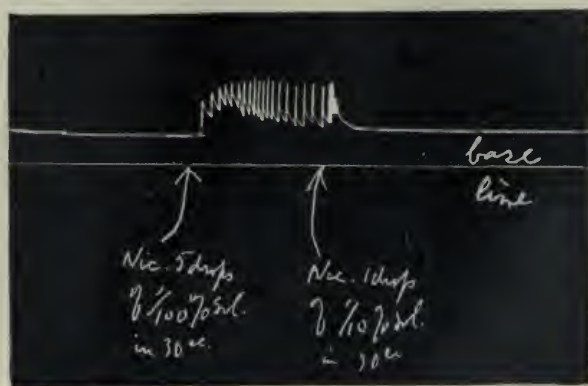


FIG. 9. Experiment January 26, 1916. Ring of pig's ureter, forty-eight hours after death of animal. Showing how the quiescent ureter is first stimulated by 0.01 per cent of nicotin and secondly, paralyzed by 0.1 per cent nicotin solution.

A similar reversal of epinephrin action was noted on longitudinal ureteral strips. The effect, however, was not so striking, owing to the lack of spontaneous peristaltic contractions in the longitudinal muscles.

#### THE ACTION OF NICOTIN

Nicotin is endowed with the well-known selective action on ganglion cells, which it first stimulates and later paralyzes. Its effect on the ureter was found to be the same as on other organs, e.g., the heart. Small doses of nicotin or nicotin salts

stimulated the contractions and increased the tonus of the ureter; larger doses, paralyzed it. This is well illustrated by the tracings (fig. 9). It was curious to note that large doses of nicotin also inhibited the action of epinephrin.

The response of the ureter to nicotin points to the existence of ganglionic structures in that organ. This agrees very prettily with the anatomic findings of Dogiel (18), R. Maier (19), and Protopopoff (2).

#### SUMMARY

1. The effect of drugs on the ureter was studied by the use of longitudinal strips and rings of the ureters of pigs and oxen.

2. The ureter when kept in Locke solution on ice retains its vitality for three days.

3. A ureteral ring preparation suspended in warm oxygenated urea Locke, exhibits spontaneous rhythmic contractions, thus affording a convenient means of studying the effect of drugs on ureteral tonus and peristalsis.

4. Epinephrin increases the rate of ureteral contractions and the tonus of the ureter, larger doses inducing a condition of tonic spasm or tetanus.

5. The action of epinephrin is reversed after previous administration of ergotoxin.

6. The effect of nicotin upon the ureter is a primary stimulating and secondary paralysis, and is a strong corroboration of the existence of ganglion cells.

7. The same results were obtained with the human ureter from a case of nephrectomy.

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## THE RÔLE OF THE LIVER IN ACUTE POLYCYTHAEMIA

### II. THE EFFECT OF EPINEPHRIN AND EMOTIONAL STIMULI ON THE RED CORPUSCLE CONTENT OF THE BLOOD IN RABBITS

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In studying acute experimental polycythaemia it has been shown by the present author (1) that it is the liver which regulates the number of erythrocytes in the blood<sup>1</sup> in the acute changes produced by the injection of epinephrin. This conclusion was reached from the following experiments.

It was found that epinephrin injected into the normal animal causes a sudden marked increase in the number of red corpuscles in the blood, this increase being of the magnitude of one or two millions per cubic millimeter, taking place in from five to fifteen minutes, and lasting about one-half hour. This increase in the number of red cells in the blood following the injection of epinephrin, takes place equally well after removing the stomach, entire intestine, the pancreas, spleen, or omentum, either singly or all at once. Excluding the liver from the circulation however excludes the production of polycythaemia following the injection of epinephrin. Furthermore injection of epinephrin causes no increase in the number of red corpuscles if the hepatic artery is ligated, the rest of the animal being left intact. But if the hepatic artery is opened, sometime after the injection of epineph-

<sup>1</sup> For the sake of brevity the terms red count, number of red cells, etc., will be used instead of the more exact term, number of erythrocytes per unit volume of blood.

rin, a marked increase in the number of red cells will take place just as if a fresh dose of epinephrin had been injected into the animal.

In carrying out further experiments along these lines, rabbits were used, as the liver may be easily removed in these animals on account of its being quite separate from the vena cava.

It was found as would have been expected, that when the liver as well as the portal organs were removed, that no polycythaemia took place when epinephrin was injected. The following protocol illustrates this point. The method used was that carried out in all of these experiments. The animal was given an anaesthetic, and the blood taken from the jugular vein by means of a needle and syringe, forced out onto a watch glass, picked up in a Thoma-Zeiss counting pipette, and counted in a Thoma-Zeiss counting chamber.

*Experiment 107.* May 22, 1915. Rabbit. Weight 1.5 kilo.

3.32 Ether.

4.00 Operation finished. Liver, stomach, entire intestine, omentum, pancreas and spleen removed.

4.03 Red count, 4,128,000.

4.04 Epinephrin 0.9 mg. per kilo intravenously.

4.20 Red count, 3,968,000.

4.40 Red count, 4,128,000.

When however normal control animals were used it was found that epinephrin here also caused no increase in the red corpuscle content of the blood, as is shown by the following experiments.

*Experiment 206.* January 29, 1916. Rabbit. Weight 2.1 kilo.

10.30 Ether. Normal control rabbit.

10.53 Red count, 5,824,000. Haematocrit reading erythrocytes, 34.0 per cent.

11.01 Red count, 6,320,000. Haematocrit reading erythrocytes, 33.0 per cent.

11.09 Epinephrin, 0.9 mg. per kilo intravenously.

11.19 Red count, 5,640,000. Haematocrit reading erythrocytes, 33.5 per cent.

- 11.32 Red count, 5,576,000. Haematocrit reading erythrocytes, 32.5 per cent.  
11.40 Red count, 4,904,000. Haematocrit reading erythrocytes, 32.5 per cent.  
11.51 Red count, 4,976,000. Haematocrit reading erythrocytes, 33.0 per cent.

*Experiment 166.* November 18, 1915. Rabbit. 2.0 kilo. Normal control rabbit.

- 9.45 Ether.  
10.03 Red count, 5,496,000.  
10.13 Red count, 5,672,000.  
10.32 Red count, 4,912,000.  
10.35 Epinephrin 0.9 mg. per kilo intravenously.  
10.53 Red count, 5,664,000.  
11.07 Red count, 5,246,000.  
11.21 Red count, 4,736,000.  
11.40 Red count, 5,040,000.

As the liver was found to be the controlling factor in epinephrin polycythaemia in dogs and cats, one would conclude from these experiments, that the liver of rabbits differs from that of these animals in response to this drug. While carrying on this work my attention was called to the very interesting experiments of Mautner and Pick (2) on the effect of "Schockgifte" and epinephrin on the liver of rabbits compared with that of dogs and cats. They found that the liver of carnivora (dogs and cats) is equipped with an extremely sensitive nervous apparatus either in the end capillaries of the portal vein or in the hepatic veins, which reacts to the injection of protein substances, or split protein substances, by a constriction of the liver capillaries which is very intensive. This can be demonstrated equally well by perfusing the livers of dogs or cats with "Schockgifte." These authors also found that epinephrin used on either the living animal or on the isolated liver, when perfused in concentration of 1:200,000 or 1:500,000, will produce the same powerful contraction of the liver capillaries. This was also found to be true of barium chloride solutions.



In herbivora however (guinea-pigs, rabbits, and monkeys), the liver reacts wholly indifferently to the injection of either "Schock-gifte" or to epinephrin in concentrations of even 1:1,000, while barium chloride causes a dilatation of the capillaries. In respect to the action of epinephrin we may speak of two classes of animals. In one as exemplified by its action on the dog, epinephrin causes a marked constriction of the liver capillaries and a polycythaemia. In the other as illustrated by the action of the drug on the rabbit, epinephrin causes no constriction of the liver capillaries, and no polycythaemia. As it has been proved that the liver controls the variation in number of red cells following the injection of epinephrin in the dog, it may possibly be considered confirmatory evidence of the importance of the liver in this mechanism of acute polycythaemia, that an animal whose liver does not respond to epinephrin, also does not respond to the injection of this substance by a change in the number of red cells per unit volume of blood.

Mautner and Pick speak of the presence of an extremely sensitive nervous mechanism in the liver of the dog, reacting to epinephrin by constriction of the capillaries, and the absence of such in the liver of the rabbit, or the presence of a much less sensitive mechanism. If such a nervous mechanism does exist, and assuming for a moment that this nervous mechanism in the liver which responds to the injection of epinephrin by a constriction of the liver capillaries, is responsible for the production of polycythaemia, one would expect certain nervous influences to cause variations in the red corpuscle content of the blood. Such has been found to be the case. It has been shown by Cohnstein and Zuntz (3) that cutting the cervical cord causes a change in the number of erythrocytes per unit volume of blood, and in experiments by the present author<sup>2</sup> it has been shown that the stimulation of certain nerves, causes great changes in the red corpuscle content of the blood, and that emotional stimuli cause the most marked increase in the number of red corpuscles obtained in any experimental conditions.

<sup>2</sup> See footnote 1.

For instance in the cat, whose normal count is between eight and nine millions, the excitement caused by allowing a dog to bark at her in one case gave a red count of 14,920,000, and in another experiment 16,776,000, both of which changes in the count occurred in less than ten minutes.

The question arises as to whether the mechanism by which this great increase in the number of red corpuscles is brought about is the same as that which regulates their number after the injection of epinephrin. That is, whether the liver controls the change in number of red cells in this instance and whether the liver is acted upon directly through nervous influences, or secondarily by the epinephrin known to be secreted by the animal in all nervous conditions which thus far have been shown to cause an increase in the number of red corpuscles.

This question of the importance of the adrenals in the production of polycythaemia is one which was briefly touched upon in a previous publication, and on which further observations will be published in the near future. It was however thought interesting in this relation to see what effect emotional stimuli would have on the red count of rabbits. We know that excitement should stimulate the output of epinephrin in these animals, but as epinephrin has been shown to cause no polycythaemia in the rabbit, emotional stimuli would cause no polycythaemia unless there were some primary nervous control of the red corpuscle content of the blood, quite independent of the presence of the adrenal glands.

The following experiments show the effect of stimuli supposed to cause excitement in the animals. Dogs were allowed to bark at the rabbits. They were subjected to loud noises, but in no case were they caused any pain, or allowed to struggle or exercise.

*Experiment 168.* November 20, 1915. Rabbit.

9.55 Red count, 7,544,000.

Emotional stimuli of excitement.

10.02 Red count, 6,920,000.

10.06 Red count, 7,176,000.

*Experiment 169.* November 26, 1915. Rabbit.

12.00 Red count, 6,680,000.

12.02 Red count, 6,754,000.

Emotional stimuli.

12.07 Red count, 6,744,000.

1.00 Red count, 6,736,000.

*Experiment 170.* November 27, 1915. Rabbit.

9.56 Red count, 5,584,000.

Emotional stimuli.

10.08 Red count, 5,824,000.

10.11 Red count, 5,664,000

It is interesting to note that two of these rabbits passed urine during the stage of attempted excitement, which might be considered to be an index of the success of the proceedings. But this urine, or that passed during the next twenty-four hours showed no reduction of Fehling's solution.

#### DISCUSSION

Excitement or the intravenous injection of epinephrin causes then no polycythaemia in rabbits. We know that even if epinephrin had been secreted into the circulation in these experiments, no polycythaemia would have been produced, as epinephrin does not cause polycythaemia in rabbits, but it is expressly pointed out that this does not mean that excitement causes no secretion of epinephrin in the rabbit. The absence of an increase in number of erythrocytes per unit volume of blood in the rabbit following a state of excitement is evidence of the fact, that no primary nervous mechanism under psychic control is present in the rabbit, capable of suddenly varying the red cell content of the blood. This may be considered to strengthen the belief that in the dog and the cat, the various physiological acute polycythaemias produced by asphyxia, excitement, exercise, etc. cause first a secretion of epinephrin and that this reacts secondarily on the liver causing an increase in number of red cells.

#### SUMMARY

1. Epinephrin in large doses (0.9 mg. per kilo intravenously) causes no increase in the number of erythrocytes per unit volume of blood in the rabbit.

2. Emotional stimuli cause no increase in the red cell content of the blood in rabbits.

3. The experiments here given indicate that the liver as a mechanism for the regulation of sudden acute changes in the number of red corpuscles in the blood varies most decidedly in its response to epinephrin and psychic stimuli in the two orders of animals, the carnivora and the herbivora. In the herbivora this organ fails entirely to respond to these agents.

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## THE PERIPHERAL POINT OF ATTACK OF STRYCHNINE

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Varrier-Jones<sup>1</sup> has demonstrated that strychnine causes an increase in capacity for muscular work with later cumulative diminution. He states "Strychnine acts essentially upon the spinal cord and medulla . . . . even in poisonous doses it does not appear to affect the cerebrum or peripheral nervous structures" and concludes that the effects are produced by a diminution of resistance to afferent impressions.

Ryan and McGuigan<sup>2</sup> confining themselves to a study of the sensory and motor neurons of the cord came to the conclusion that strychnine action was mainly of the nature of increase in irritability of either sensory or motor cells or an intercalated cell. In a later communication, McGuigan and Becht<sup>3</sup> state that "Strychnine acts on both motor and sensory neurons and no tetany can develop from its action unless the motor neuron is directly acted upon by it." Dusser de Barenne,<sup>4</sup> whose work was carried on independently of McGuigan and practically at the same time, also arrived at the conclusion "that only the combined poisoning of the dorsal and ventral spinal cord mechanisms induces a typical strychnine tetanus," and that "there is no justification for speaking of an elective action of strychnine upon a dorsal sensory coördinating mechanism in the spinal cord."

<sup>1</sup> Varrier-Jones: *Journal of Physiol.*, xxxvi, no. 6, p. 435, 1907-08.

<sup>2</sup> Ryan and McGuigan: *Journ. Pharm. Exp. Ther.*, ii, no. 4, p. 319, 1911.

<sup>3</sup> McGuigan and Becht: *Journ. Pharm. Exp. Ther.*, vi, no. 5, p. 469, 1914.

<sup>4</sup> Dusser de Barenne: *Folio Neuro-Biologia*, v, p. 42, 1911.

Sherrington<sup>5</sup> in a study of the action of strychnine on the reflex arc showed that this drug causes a release from inhibition and consequent destruction of coördination.

Richet<sup>6</sup> demonstrated conclusively that strychnine in large doses has a curare-like effect when the animals are kept alive by artificial respiration. This leads to the conclusion that strychnine has a peripheral point of attack, but leaves open the site of action and the type of response of the peripheral structures to small doses.

From the work of Koch and Mostrom<sup>7</sup> it is evident that some sort of a combination, probably chemical, takes place between strychnine and the lipins supposed to be an integral part of nerve structure.

The cumulative evidence cited assigns rather definitely to strychnine the function of, in some way or other, facilitating the passage of the nerve impulse from one neuron to the other. That is to say it increases the irritability of the transmitting substance.

Admitting that there is a substance interposed between the endings or beginnings of the neurons in the cord, that in some way modifies the nature of the transmitted stimuli, and that strychnine has the power to disturb this property leading to uncontrollable muscular movements, and admitting that the reaction is of the nature of a chemical union between substance and strychnine, is it illogical to suppose that it may also have a similar action upon the "receptive substance," of Langley<sup>8</sup> at the neuro-muscular junction and lead to a facilitation of discharge of nerve impulse through an increased irritability of this substance?

Experimental evidence seems to support the view that the seat of fatigue in a nerve-muscle preparation lies in the neuro-muscular junction. In an isolated preparation of this sort, there is available but a certain amount of energy producing

<sup>5</sup> Sherrington: *Journ. of Physiol.*, xxxvi, no. 2-3, p. 185, 1907-08.

<sup>6</sup> Richet: *Comptes Rendes de l'Acad. d. Sci. Paris*, xci, p. 131, 1880.

<sup>7</sup> Koch and Mostrom: *Journ. Pharm. Exp. Ther.*, ii, no. 3, p. 265, 1911.

<sup>8</sup> Langley: *Journ. of Physiol.*, xxxvi, no. 4-5, p. 347, 1907-08.

material. Hence by an increased irritability of the neuro-muscular junction, but two effects can be obtained, when comparing a strychninized muscle with one not so having been drugged. Either the onset of fatigue is delayed and the muscle has a greater capacity for utilizing the available energy producing material, or the transmitted stimuli are individually more effective and there is made evident a primary increase in work capacity with a quicker "Cumulative depression."

With direct stimulation of muscle the question of the action of strychnine as affecting muscle tissue irritability is brought in. It may be supposed that if strychnine increases the irritability of both muscle tissue and neuro-muscular junction, that the resultant effect of direct stimulation would be the algebraic sum of the two factors. It is then obviously impossible to determine the action, if any, of strychnine upon muscle tissue as such, when the neuro-muscular connection is functioning, for any changes observed as the result of the strychnine action can be ascribed to the action of this drug, either upon the receptive substance or the muscle tissue, or both.

There are two elementary methods for eliminating the nerve control of muscle; the one is accomplished by so poisoning the receptive substance by some drug that the transmission of impulse through the substance is prevented; or by cutting the nerve to the muscle and studying the effects after degeneration.

The specific action of curare in blocking the passage of nerve impulse from fibre to muscle is too well known to need discussion.

If we utilize this drug and administer strychnine after the effects of the former have come to their maximum, it is plausible to expect an increase in irritability of muscle tissue if the latter drug has any direct stimulative action upon muscle tissue as such.

With these premises in mind, experiments were planned to determine the following points:

First, Does strychnine increase the irritability of muscle tissue itself, apart from its action on the neuro-muscular junction?

Second, Does strychnine affect the transmitting substance between nerve ending and muscle as it does the synapse in the



cord leading to a facilitation of passage of nerve impulse, consequent fatigue delay, and increase in irritability of the nerve muscle system?

#### SERIES I

In this series pithed frogs were poisoned by the injection of four drops of a 1 per cent solution of curare into the dorsal lymph sac. When paralysis was complete, as evidenced by inhibition of nerve impulse with consequent non-contraction of muscle, the vessels of one leg were ligated and the gastrocnemius removed and made to record its fatigue curve, while the other muscle was subjected to the action of 0.8 cc. of a 0.1 per cent strychnine solution, injected into the dorsal lymph sac and al-

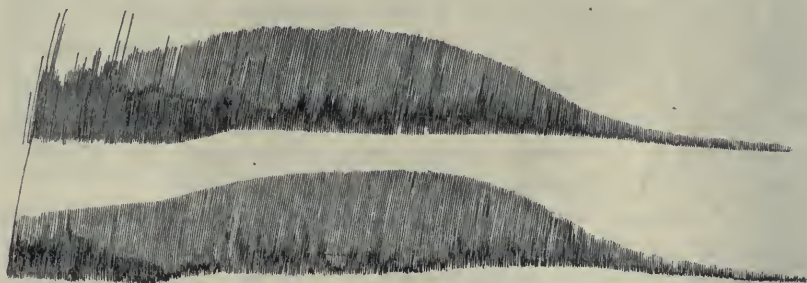


PLATE I. Top tracing: Curarized muscle. Bottom tracing: Strychninized curarized muscle. Direct muscle stimulation.

lowed ten minutes for absorption. The curarized strychninized muscle was then made to write its fatigue curve and the results compared. Control experiments were made in which the fatigue curve was first traced by the curarized strychninized muscle and followed by a similar curve traced by the curarized non-strychninized muscle. Both methods of procedure yielded similar results. The gastrocnemii were removed from the distal attachments but remained connected with the femur and were so set up as to record their fatigue curves upon a slowly moving drum, when directly stimulated by maximal break induction shocks repeated at two-second intervals regulated by an electrical metronome. The weight lifted exclusive of the lever amounted to forty-five grams. In so far as possible the

factors of strength of stimulus, load, temperature, etc., were kept constant.

Plate I represents tracings obtained in this manner. The upper curve was traced by the curarized non-strychninized muscle, the lower by the curarized strychninized muscle. These tracings are typical and demonstrate that within the limits of the experimental procedure herein outlined strychnine does not, to any marked extent, increase irritability of muscle tissue, if at all.

#### SERIES II

Frogs having their brains pithed were treated as follows. The sciatic nerves of both legs were exposed, taking care not to injure blood vessels or muscles. The cut nerves were supported upon a glass rod and the whole kept moist with normal saline. The subminimal induced current stimulus was obtained for each nerve through separate inductoria and separate sources of current. The vessels of one leg were ligated and 0.8 cc. of a 0.1 per cent solution of strychnine injected into the dorsal lymph sac. Allowing ten minutes for absorption, the individual stimuli were applied and it was found that whereas the muscles deprived of their blood supply and consequently removed from the action of strychnine still failed to respond to the sub-minimal stimuli applied through the nerve, the muscles to which the blood supply had been uninterrupted now responded by contraction to the stimuli which had previously been subminimal. Hence under the influence of strychnine the receptive substance which had previously offered sufficient resistance to the passage of the impulse set up in the nerve fibre by the sub-minimal current to prevent its transference to the muscle, with resulting contraction, now permitted the passage of this impulse and the muscle contracted.

It is therefore demonstrated that strychnine has the power of diminishing the resistance to the passage of a nerve impulse through the receptive substance and this facilitation can be considered an increase in irritability, which leads to the idea that the response of a muscle according to its function, to a stimulus

through its nerve, depends not so much upon the muscle tissue itself as upon the condition of the receptive or transmitting substance interposed between nerve ending and muscle tissue.

### SERIES III

In this group of experiments an attempt was made to record graphically the results of the previous series. The gastrocnemius was removed from frogs having had the brains pithed, care being taken to prevent undue loss of blood. This relatively normal muscle was made to record its fatigue curve when stimulated through its nerve by maximum break induction shocks repeated at two-second intervals. In the meantime 0.8 cc. of

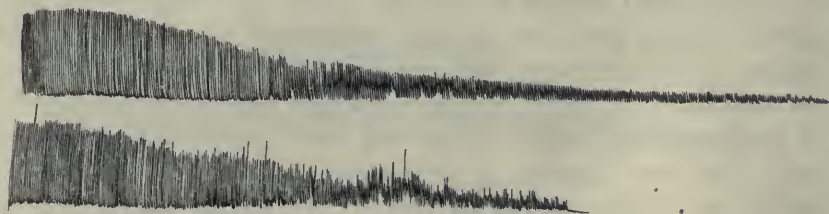


PLATE II. Top tracing: Strychninized muscle. Bottom tracing: Normal muscle. Stimulation through nerve.

a 0.1 per cent solution of strychnine was injected into the dorsal lymph sac of the frog and after allowing ten minutes for absorption the remaining gastrocnemius was removed and a tracing made of its fatigue curve under similar condition.

Plate II shows conclusively that the onset of fatigue is delayed and that the muscle is capable of doing more work. This is not due to increased liberation of energy producing material, but is due to the decrease in resistance or facilitation of the passage of the nerve impulse through the receptive substance.

This then is graphic evidence that strychnine facilitates the passage of the nerve impulse from nerve fibre to muscle and thus by making available the otherwise non-utilized energy producing material delays the onset of fatigue.



## SERIES IV

In Series IV the problem was attacked from the point of direct muscle stimulation other factors of technique remaining the same.

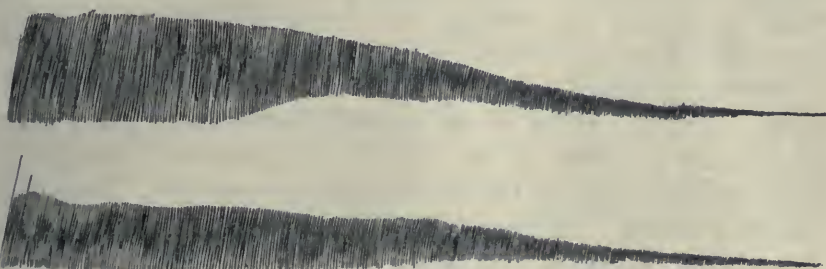


PLATE III. Top tracing: Strychninized muscle. Bottom tracing: Normal muscle. Direct muscle stimulation.

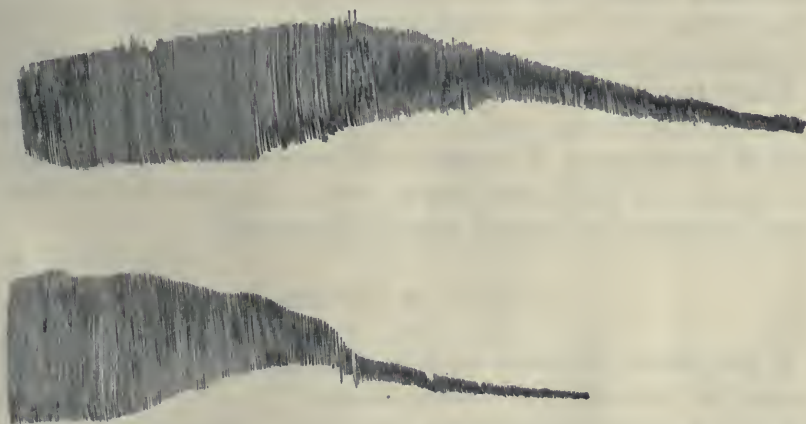


PLATE IV. Top tracing: Normal. Bottom tracing: Strychninized. Direct muscle stimulation.

On Plate III the upper tracing represents the effect of strychnine, the lower tracing being the norm. On Plate IV the arrangement is reversed.

An initial increase in irritability is evident. There is no evidence of any increase in total work done. The initial increase in irritability necessarily implies an initial increase in capacity



for work with more rapid onset of fatigue, due to the self-evidenced fact that in isolated preparations of this sort only a certain amount of energy producing material is present and by direct muscle stimulation this can be utilized, hence if more energy is expended at the beginning the utilization of the material producing this energy will be more rapid with earlier onset of fatigue as a consequence.

Now in stimulation of this type two factors are concerned. First, the direct effect of the stimuli upon muscle tissue itself and second the effect of the stimuli as transmitted through the receptive substance of the neuro-muscular junction. From the results of Series I, the first factor may be considered a constant. Any change in the second factor tending towards a decreased resistance to the passage of the stimulus would result in a facilitation and consequent increase in strength of effective stimulus. This then would develop in increased height of contraction, other things remaining equal, meaning an initial increased expenditure of energy or an initial increase in work capacity.

From the recorded tracings, it is evident that strychnine produces this change, and as a result there is obtained facilitation of transmission of impulse with consequent initial increase in capacity for work with earlier onset of fatigue due to more rapid utilization of energy producing material.

#### SUMMARY OF RESULTS

1. Strychnine injected into a curarized frog does not increase the irritability of muscle tissue.

2. In a strychninized frog previously subminimal stimuli become effective when applied to a nerve connected with its muscle.

3. When the muscle of a strychninized frog is stimulated through its nerve the onset of fatigue is delayed and more work is accomplished.

4. When the muscle of a strychninized frog is directly stimulated there is an initial increase in capacity for muscular work with earlier fatigue. The total work done is not changed.

## CONCLUSIONS

The site of the peripheral action of strychnine is the receptive substance of the neuro-muscular junction. Its action here is similar to its action on the synapses in the cord leading to a decrease in resistance to the passage of the nerve impulse and a facilitation of its transmission across the junction. As a result of this there is normally a more efficient utilization of the material present serving as the source of muscular energy. There may be an initial increase in the capacity for work but this is not due to any increase in irritability of muscle tissue. Muscle tissue itself is not increased in irritability by strychnine.



# ARTIFICIAL CEREBRAL CIRCULATION AFTER CIRCULATORY ISOLATION OF THE MAMMALIAN BRAIN

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This investigation was started during the early part of the year 1914 with the object in view of studying the effect of certain agents upon the medullary centers.

Heretofore, it has been customary in many cases to determine this point either by destroying portions of the central nervous system or by severing the nervous connection with the various organs of the body.

It occurred to me that it was possible to reverse this order and determine the effect of those agents acting upon the medullary centers, with the whole nervous system intact, which could be accomplished by carrying on an artificial circulation through the brain after it had been isolated from the systemic circulation.

A number of experiments had been performed before any literature bearing on the subject could be found, but in my search it was discovered that in 1877 François Franck<sup>2</sup> in his "Investigations on the Effect that Variations in Intra-cranial and Intra-cardiac Pressure Exert on the Rhythm of Heart-beats" had ligated the vertebral arteries and veins below the atlas and introduced a bifurcated cannula into the cephalic end of the carotids which was connected with a reservoir of defibrinated blood under a constant pressure of 140 mm. of mercury. The blood was perfused through the vessels of the brain and allowed to escape through the jugulars which were equipped with small cannulae.

<sup>1</sup> A part of the expense of this research was defrayed by a grant from the research fund granted by the Regents of the University.

<sup>2</sup> Physiologie Experimentale, 1877, Travaux du Laboratoire de M. Marey.



In order to render the circulation of the body and brain absolutely independent of each other, he sectioned the medulla below the bulb, and placed a tourniquet tightly around the neck, leaving free only the carotids, jugulars and the vagi. The animal was sustained by artificial respiration.

Hayem and Barrier<sup>3</sup> in 1887 perfused blood through the head of decapitated dogs using blood from the same animal, or horse blood, in order to determine whether the higher centers of the brain might be revived. In their communication they report that Brown-Sequard had performed similar experiments thirty years previously, but no report could be found in the literature. At an earlier date J. V. Laborde<sup>4</sup> had performed the same experiment perfusing decapitated human heads and also that of animals. The experiments of Hayem and Barrier and Laborde were undertaken with an entirely different object in view and have no bearing on the present experiments.

The experiment of Francois Franck, although somewhat of the same order, is essentially different from the method I have employed. In his experiment, the cord has been severed, which is not only conducive to shock, but all nervous connection between body and brain, with the exception of the vagi, has been destroyed. Eisenbrey<sup>5</sup> describes a method of isolating the cerebro-medullary circulation of the dog in which the chest was opened and the large vessels at the base of the heart were ligated. The cerebro-medullary circulation was maintained by transfusion from a second dog which served as donor, and artificial respiration was employed to sustain life.

He found that an injection of horse serum into the cerebral circulation of a sensitized animal did not produce the usual fall of blood pressure as characteristic of anaphylactic shock, and this was taken as evidence of a successful isolation.

In order to determine the thoroughness of the isolation of the brain and medulla from the systemic circulation, as well as the effect of the drug upon the medullary centers, I employed

<sup>3</sup> Comptes Rendus Acad. de Sci., Paris, vol. 104, p. 272.

<sup>4</sup> Revue Scientifique, 1884 and 1885.

<sup>5</sup> Scientific Proceedings Society for Exp. Biol. and Med., vol. 7, p. 113.

epinephrin which was perfused through the brain in varying concentrations with little or no effect on blood pressure, while the same doses introduced into the femoral vein gave the characteristic rise.

At the end of the experiment, a concentrated solution of methylene blue was perfused through the cephalic vessels of a number of animals, after which the animal was autopsied. At autopsy there is evidence that some of the stain reaches the general circulation from the fact that the intima of the aorta is more or less blue and some of the intercostal vessels when severed are found to contain some of the stain. The abdominal viscera in most instances was free from the stain but in a few dogs it could be detected. In order to exclude the possibility of the stain being carried to the systemic circulation through anastomosing branches of the suprascapular or intercostal vessels, all the muscles of the neck were completely severed down to the vertebra in one animal (dog 24).

After perfusing with methylene blue the stain is found in the aorta, internal mammary and intercostal vessels as in the preceding animals. This leads one to conclude that the spinal arteries are in the main the channels through which the stain must have traveled to reach the general circulation, and it would be difficult to say how much might have escaped through this channel. Owing to the concentration of the methylene blue a very small amount could have easily produced the stain.

From the fact that epinephrin in fairly strong solutions perfused through the cerebral circulation gave an effect which was comparatively small or absent, as well as with the evidence presented by Eisenbrey in his experiments, would warrant the conclusion that the isolation was fairly complete although not absolute.

In the experiments thus far performed, 23 dogs weighing from 15 to 50 kg. were employed. They were anaesthetized by giving 0.010 per kilogram of morphine sulphate hypodermically, followed in twenty to thirty minutes by 8 cc. per kilogram of Gréhant anaesthesia.

*Method.* The external jugular veins were exposed and a loose ligature placed around them so that they may easily be picked up when wanted, likewise the carotids. The internal jugular veins were ligated at once.

The vertebral artery and vein on the left side were next exposed below the sixth cervical vertebra, the vein ligated and a cannula introduced into the artery.

The cannula is bent U-shaped, one of the limbs being much shorter than the other, the short end being the one which is tied into the artery. The right vertebral artery and vein were next sought and a strong ligature thrown around both, so that they may be tied with the same ligature at the proper time. A cannula was placed in the trachea to which may be attached a tambour for recording respiration or a tube from the bellows in case artificial respiration is found necessary.

A cannula was placed in the cephalic end of the right carotid, also the cardiac end of the same artery. The cannula in the vertebral artery was then filled with 0.9 per cent salt solution, a feather being employed to work out all the air. It was then connected with the cannula in the cephalic end of the carotid, a combination Y and T tube being placed between the two cannulae. The combination tube was made by attaching a piece of glass tubing to the stem of a U shaped tube, so that it is a combination U and T tube (*a*). Plate I.

There is no particular object in view in connecting the left vertebral with the right carotid, except to afford a little more space for cannulae and connections. The cannula in the cardiac end of the carotid was then connected to the manometer for blood pressure tracing.

One limb of the U (*b*) of the combination U and T tube was then connected with the perfusion apparatus and to the other (*c*) a rubber tube was attached which serves for the escape of air that fills part of the apparatus, after which it is closed by a pinch clamp. The perfusion apparatus consists of a round galvanized iron pan 14 inches in diameter and 6 inches deep in which are fastened two 1500 cc. and one 500 cc. three-necked Woulff bottles. These bottles fit into brass frames



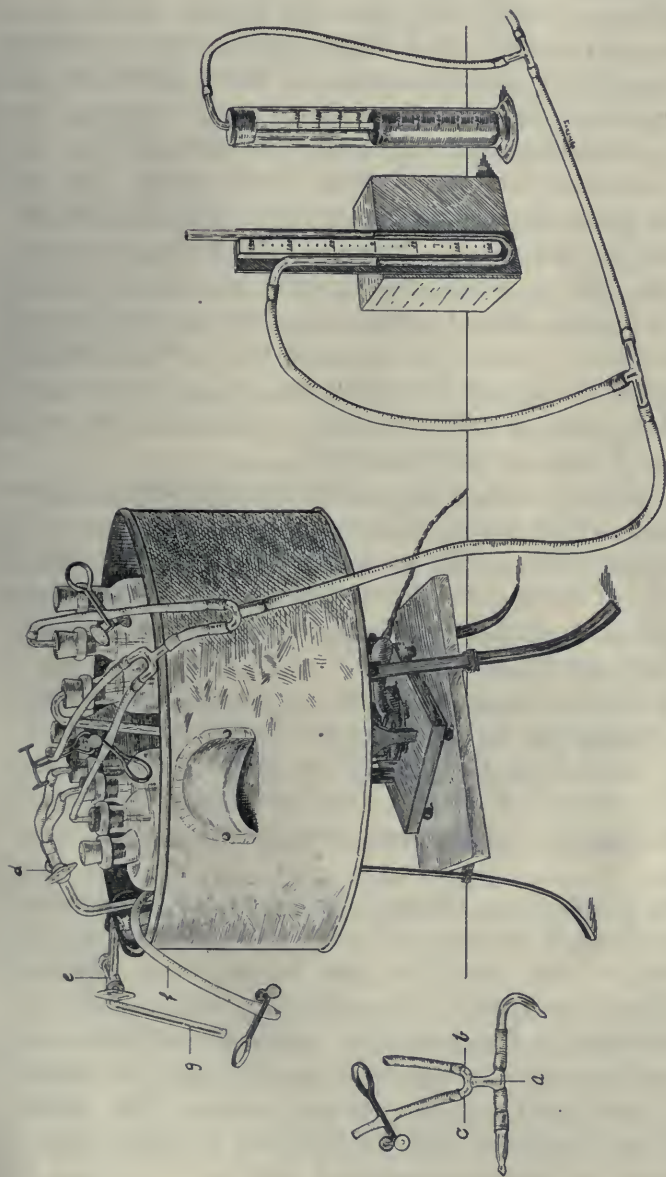


PLATE I. PERSPECTIVE VIEW OF PERFUSION APPARATUS

*a*, Combination U and T tube; *b*, limb of U tube which is connected with free end of three-way cock; *c*, limb of U tube for the escapement of air; *d*, three-way cock; *e*, three-way cock; *f*, outlet tube for draining 500 cc. bottle.



which are attached to the bottom of the pan and hold the bottles firmly, about one-half inch from the bottom of the pan. Into one of the side necks of the 1500 cc. bottles is fitted a rubber stopper through which passes a glass tube barely touching the bottom of the bottle, the tubes from the two bottles being connected with a three-way cock (*d*) so that the flow of fluid can be instantly changed from one bottle to the other. To the free end of the three-way cock is connected one tube of a second three-way cock (*e*) and to the other the tube leading from the side neck of the 500 cc. Woulff bottle which also has a Y tube intervening, a rubber tube (*f*) being attached to one limb of the Y for the outlet of air, or for draining the bottle if desired.

The free end of the second three-way cock (*g*) is connected by means of a short piece of rubber tubing to one limb of the combination U and T tube already described.

The central neck of each of the bottles is fitted with a rubber stopper through which passes a glass tube reaching nearly to the bottom of the bottle. This serves for the air pressure which was employed for forcing the fluid through the outlet tubes. The other side neck of each bottle is provided with a stopper and is employed for filling.

The pan is filled with water nearly to the top and kept at the desired temperature by means of an electric hot plate.

The outlet tubes from the bottles dip under the water and coil around the bottom of the pan, thus facilitating the keeping of the circulating fluid at the same temperature as the water in the pan.

The perfusion apparatus having been placed in position and properly connected, a cannula is placed in the jugular vein on either the right or left side (the right having been employed in all the earlier experiments), through which 100 to 750 cc. of blood was withdrawn and defibrinated. A corresponding amount of 0.9 per cent solution of sodium chloride or Ringer-Langendorff solution was introduced into the femoral artery. The defibrinated blood was diluted to 2000 or 6000 cc. with 0.9 per cent sodium chloride or Ringer-Langendorff solution.

The 1500 cc. bottles were filled with the diluted blood and the air driven out of the tubes, the 500 cc. bottle which is intended for the introduction of any drug that may be desired, was filled with blood solution and the air expelled.

A tambour was connected with the tracheal cannula for recording respiration, a T tube being introduced near the cannula to permit the breathing of fresh air.

The clamps are now removed from the cervical end of the carotid and the vertebral arteries and the jugular vein, the air pressure turned on and the three-way cock turned so that the dilute defibrinated blood flows through the vessels of the brain and medulla.

The air pressure is regulated by means of an escape tube dipping under mercury and a mercury manometer is also connected to a side outlet in the air tube, so that the pressure may be accurately measured in millimeters of mercury.

The right vertebral artery and vein are ligated with the single ligature already in place, likewise the left carotid artery and the free jugular vein.

All direct circulation between the brain and the medulla and the body is now shut off except that which occurs through the spinal arteries and veins. The circulating fluid now enters the right carotid and left vertebral arteries and flows out through either the right or left external jugular vein as the case may be.

It was considered important from the anatomical standpoint that part of the circulating fluid should enter a vertebral artery in order that a generous flow might be kept up through the basilar artery which gives off transverse arteries supplying the pons and medulla.

In the first two experiments, the pressure necessary for forcing the fluid through the arteries was furnished by the use of suspended mercury bulbs, but this was abandoned on account of being inconvenient, and there was no means of keeping the perfusion fluid at a uniform temperature and of oxygenating the solution.

The result, however, showed the experiment was feasible, for the animals were able to breathe without assistance for a period of forty-three and forty-seven minutes respectively. The apparatus previously described was then constructed and compressed oxygen employed as a source of pressure which was thought to be of advantage in order to better oxygenate the solution.

The only available oxygen tank having been imperfectly closed at the end of the fourth experiment, allowed the escape of the contents, so compressed air, which was available, was employed. This appeared to work as satisfactorily as the oxygen, so it has been employed in all the rest of the experiments.

In comparing the respiratory tracings, it was noticed that in some cases there are long pauses or the respiration may be very much slowed, which is probably due to a depression of the respiratory center since it is usually revived by adding to the perfusion fluid, a drug which stimulates respiration. It was also noticed that the defibrinated blood solution, although as dilute as ten to one hundred was of a venous color as it flowed from the jugular vein, and it had the same tendency to clot as fresh blood. It was therefore necessary to whip the blood solution to oxygenate, as well as to defibrinate, each time it was forced through the cerebral vessels.

In one experiment (dog 19) defibrinated beef blood was employed but found impracticable owing to an almost instantaneous intravascular clotting.

The part of the experiment which appeared most interesting was the fact that under favorable conditions the animal was able to breathe for a considerable period of time, showing that the respiratory center was active although subjected to a most unnatural source for blood supply and nourishment.

A table is here given showing the length of time the respiratory center was active after the brain had been isolated and the artificial circulation commenced.

DOG	MINUTES	REMARKS
5	43	
6	47	
7	3	Air entered vessels.
8	35	
9	15	
10	19	
11	38	
12	33	
13	100	
14	2	
15	89	
D16	0	Ceases as vessels are ligated.
17	0	Ceases as vessels are ligated.
18	5	Isolation incomplete.
19	1	Beef blood. Intravascular clotting at once.
20	5	Air entered vessels.
21	6	Stopped by epinephrin.
22	12	Stopped by epinephrin.
23	11	
24	1	
25	22	
27	86	
28	16	

Since this result could be obtained upon a medullary center which is so easily paralyzed and which under the conditions of the experiment shows unmistakable evidence of its activity, it is natural to assume that the other centers in the medulla are equally as active, and should respond to the actions of the various agents which are capable of producing either stimulation or depression; and this was found to be the case.

A number of drugs have been employed besides the effect of temperature, pressure and air. While the experiments of any series are too few to allow the drawing of deductions, it may be stated that in three of the dogs (7, 12, 16), where air accidentally entered the vessels there was a very marked rise in blood pressure, probably from stimulation of the vasomotor center. On another dog (6) where the temperature of the perfusion fluid was increased, there also occurred a marked rise in blood pressure. The limited amount of blood available in a



few of the experiments necessitated the employment of pure 0.9 per cent solution of sodium chloride or Ringer-Langendorff solution at times when the perfused blood solution was being prepared for reintroduction. The effects have been rather variable; in some cases there are no changes produced, but in most instances there is a stimulation of respiration accompanied by a rise in blood pressure. Occasionally, there is an apparent vagus stimulation.

It is hoped that the method here reported will, under ideal conditions, prove of value in studying some of the problems in which the medullary centers are primarily concerned.

In conclusion, I desire to thank Dr. R. A. Hall and the various students who have so willingly assisted me in making the experiments possible.

## OBSERVATIONS ON THE EFFECT OF EPINEPHRINE ON THE MEDULLARY CENTERS

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The slowing of the heart beat commonly observed when epinephrine is introduced into the circulation is almost universally held to be due to an excitation of the vagus center, which is induced by the rise in blood pressure, and therefore is an indirect effect.

This view is supported by the well known fact that if the blood pressure be reduced by bleeding during the epinephrine rise, the slowing disappears at once.

The question as to whether epinephrine has any direct action upon this center is still unsettled, and there is but little experimental evidence reported in the literature which would suggest that the drug might produce a direct stimulation.

Biedl and Reiner<sup>2</sup> in a series of experiments found that when extract of the suprarenal gland was injected into the cephalic end of the carotid artery, there was a slowing of the heart of brief duration before there was any rise in blood pressure. They hold that this effect is due to a direct stimulation of the vagus center, for this is followed by a period during which the heart rate is normal. During the rise in blood pressure there is a second slowing which is accounted for by the indirect action.

Gerhardt<sup>3</sup> employing small doses of epinephrine finds the vagus effect occurs when the blood pressure is raised 20 to 30 mm. of mercury but he thinks that the slowing is due to the

<sup>1</sup> A part of the expense of this research was defrayed by a grant from the research fund granted by the Regents of the University.

<sup>2</sup> Arch. für die ges. Phys., vol. 73, p. 385.

<sup>3</sup> Schmiedeberg Arch., vol. 44, p. 161.

rise. He reports a few cases where the slowing was so great that there was a fall in blood pressure. He also points out the fact that the vagus effect does not occur in all cases.

The present investigation was undertaken with the hope that some additional facts might be discovered which would aid in solving the problem.

The experiments herein reported were performed on 22 dogs by employing the method as described in the previous paper. The perfusion fluid consisted of defibrinated dog blood diluted with 0.9 per cent sodium chloride, which was used in the first 5 experiments; in the others the dilution was made with Ringer-Langendorff solution. The amount of blood present in the perfusion solutions varied considerably, since it was furnished by the animal upon which the experiment was performed. As there was some disturbance in blood pressure at the time when the vessels were ligated and the perfusion commenced it was thought advisable to tabulate the results to see what bearing they might have on the problem.

The result of the tabulation shows that the average blood pressure of the 22 animals before perfusion was commenced, was 76.4 mm. of mercury.

The average maximum pressure after the perfusion had been started, was 98.2 mm.

The average rise in blood pressure for the 22 animals amounts to 21.8 mm., which is about the minimum pressure at which Gerhardt observed the vagus effect.

In 11 of these animals there was no change in heart rate, while in 9 there was an increase. In two of the animals (dogs 16 and 21) there was a slowing of the heart due to a vagus stimulation which was of brief duration. An initial blood pressure of 60 mm. in the case of dog 16 was raised to only 62 mm. while the heart rate dropped from 80 beats per minute to 56 beats.

In dog 21 the blood pressure rose from 56 mm. to 68 mm. while the heart rate was slowed from 76 beats per minute to 64 beats.

In neither of these animals could one say that the stimulation was due to a rise in blood pressure, for the rise was not sufficient to account for the result. From the fact that none

of the other animals showed any such effect, and in some there was a considerable rise in blood pressure, it is possible that the vagus center was stimulated directly as was also the vasomotor center.

*Effect of epinephrine upon the vagus center.* The effect of epinephrine was tried upon 14 animals of the series with somewhat variable results.

The epinephrine was added to the perfusion solution immediately before being used in order to preclude the possibility of decomposition. The epinephrine content in the solutions varied in strength from 1 drop in 750 cc. of the blood solution to 5 drops in 200 cc. It was observed that the weaker solutions were the more effective.

The most marked effect where slowing of the heart occurred, was obtained when the dilution was 2 drops in 500 cc. to 750 cc. It was also observed that as a rule the first time the drug was perfused the effect was more pronounced than in the subsequent perfusions. Epinephrine employed in this manner apparently produces a condition in which the vagus center does not respond to the action of the drug after it has been employed a few times.

This does not appear to be a paralysis of the vagus center for there is no increase in heart rate.

Verworn<sup>4</sup> in his conclusions from a series of experiments states that suprarenal extract may in certain doses produce a transitory condition in which the vagus center does not respond to stimulation.

Kahn<sup>5</sup> shows that the depressor reflex is abolished by suprarenal extract and arrives at the same conclusion as Verworn, that the vagus center is depressed.

A tabulation of the results obtained on 14 dogs where epinephrine had been introduced shows that in 9 of these there was a slowing of the heart, while in 5 it was absent. The average blood pressure of the 9 dogs before perfusing the brain with the solution to which epinephrine had been added was 81.1 mm. The average blood pressure taken during or immediately fol-

<sup>4</sup> Arch. für Phys., 1903, p. 65.

<sup>5</sup> Ibid., 1903, p. 522.



lowing the perfusion was 75.1 mm. It will be observed that the average blood pressure for the 9 dogs shows a fall. In only one animal (dog 13) was there a rise of any significance. In this case the blood pressure rose from 132 mm. to 156 mm. at the same time the heart rate dropped from 148 beats per minute to 60 beats.

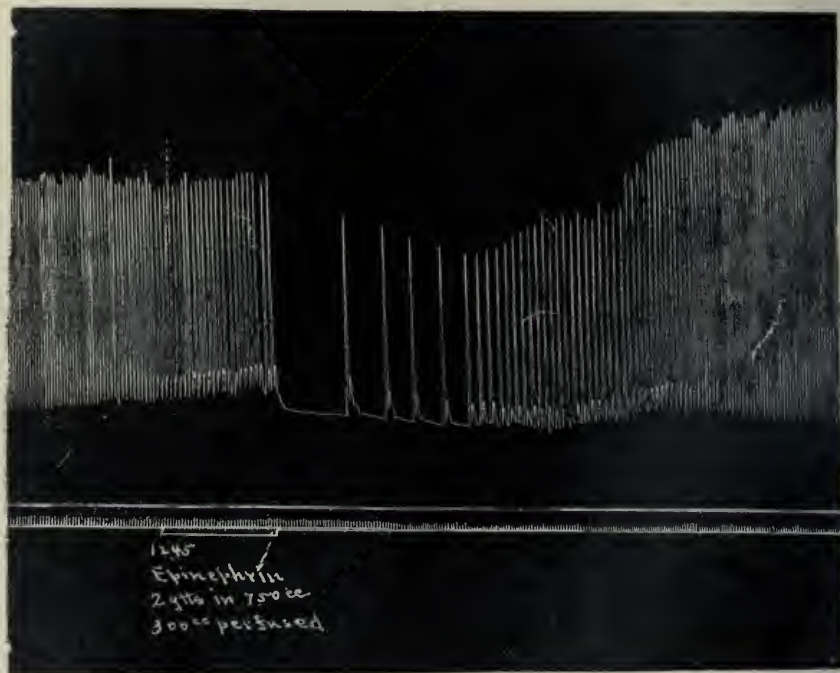


FIG. 1. Shows slowing of the heart due to vagus stimulation produced by perfusing epinephrine through the cerebral vessels.

These results tend to show that there was a central vagus stimulation which was not brought about by a rise in blood pressure.

As evidence that the drug has acted directly upon the vagus center it will be observed (fig. 1) that the slowing of the heart began at the moment the perfusion was started and it cannot be said that it is in any way connected with a rise in blood pressure.

It might be assumed that it was a secondary effect due

to a congestion of the cerebral vessels resulting from a vaso-constriction.

This possibility cannot be absolutely excluded, but it seems hardly probable since the perfusion fluid entered the vessels under a constant pressure. Even though the outflow was retarded there could be no rise in pressure since the mercury valve prevented the pressure rising above the point at which it was set; usually about 90 mm. of mercury. Again it might be said that the vagus excitation was due to an anaemia resulting from a vaso-constriction of the cerebral vessels.

This assumption, however, is not in accord with the observation that the weaker solutions gave the most marked effect.

The results obtained in my experiments, where a method entirely different from that of other workers on the problem has been employed, tend to confirm some of the observations reported by other investigators. It is quite conceivable that the vagus effect observed by Gerhardt where small doses of the drug were employed and produced a rise in blood pressure of from 20 to 30 mm. of mercury, could have been in part due to a direct stimulation of the vagus center. This appears all the more probable from the fact that he observed cases where the vagus stimulation was so great that the blood pressure fell.

My experiments confirm his findings in that the vagus effect is not obtained in all cases.

It may be stated, as pointed out in the previous paper, that there was not an absolute isolation of the cerebral circulation from that of the body. The spinal arteries and veins were still intact and there is some collateral circulation between the vessels of the head and neck and those of the shoulders. In order to eliminate the collateral circulation, the muscles of the neck were all severed down to the cervical vertebrae on dog 24, and a tourniquet was placed tightly about the neck exclusive of the vagi, trachea and carotids on dog 28. The results obtained on these animals did not appear to differ from those observed on the others.

In dog 24 epinephrine caused a slight rise in blood pressure accompanied by a slowing of the heart.

In dog 28 there was a rise in blood pressure while the heart rate remains unchanged. The amount of epinephrine carried through the spinal vessels must have been very small and it is hardly probable that it was sufficient to account for the rise in blood pressure.

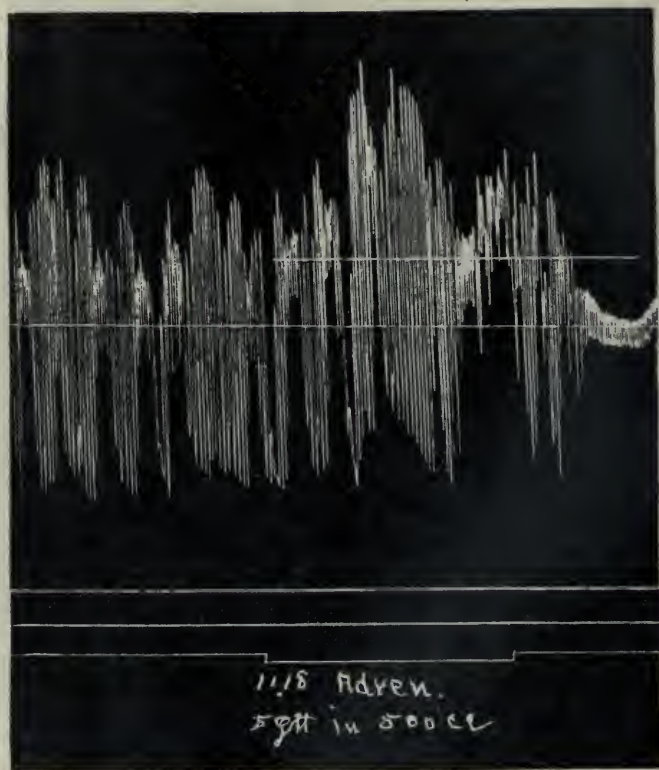


FIG. 2. Showing the rise in blood pressure produced by perfusing epinephrine through the cerebral vessels where the spinal vessels are the only vascular connection between the body and the brain.

Figure 2 taken from dog 28 shows a rise in blood pressure where only the spinal vessels could have carried the drug to the systemic circulation.<sup>6</sup>

<sup>6</sup> The heart beat was very erratic in this animal, but this condition was present before either the critical part of the operation or the perfusion was begun, and continued during the whole period of the experiment.



It was also observed that the amount of the rise in blood pressure was not governed by the adrenaline content in the perfusion fluid.

In fact the rise produced by a solution containing a small amount of the drug, and where the same animal was employed, was sometimes greater than it was where the solution contained a larger amount. Figure 3 shows that 2 drops of epinephrine

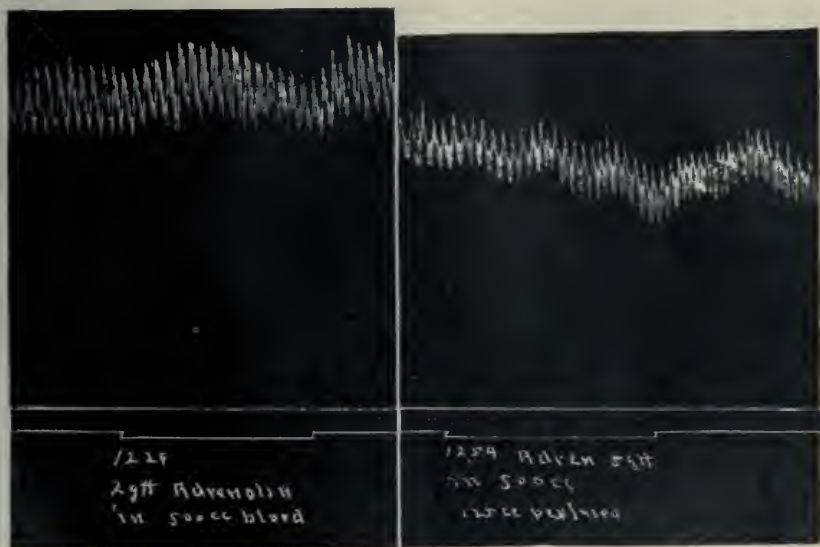


FIG. 3. Showing that the weaker solution of epinephrine produces a rise in blood pressure while the stronger one produces a fall. The respiratory tracing which was taken at the same time shows no change in either case.

in 500 cc. of the perfusion fluid produced a greater rise than did 5 drops in the same amount of fluid.

In none of the experiments was the rise in blood pressure very pronounced although it was usually but not always present, if not overshadowed by the vagus effect. Occasionally there was a lowering of blood pressure without any slowing of the heart, and this effect was more apt to occur when the perfusion fluid contained the larger amounts of the drug.

In order to determine what effect the perfused epinephrine blood might have when introduced into the systemic circulation,



one drop of epinephrine was introduced into the femoral vein of one animal (dog 27), which caused the blood pressure to rise 22 mm. of mercury.

One hundred and twenty-five cubic centimeters of blood containing 1 drop to each 100 cc. was then perfused through the brain which caused a rise of 10 mm. of mercury. The 125 cc. of the perfused blood was then introduced into the femoral vein which caused a rise of 24 mm. of mercury. Figure 4.

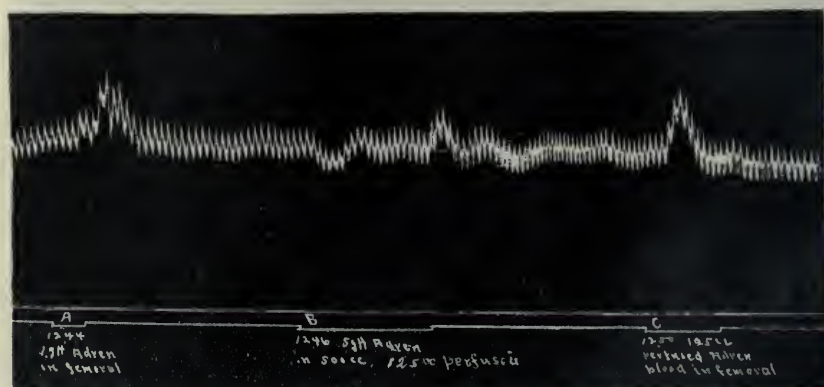


FIG. 4. A, one drop epinephrine injected into the femoral vein; B, 125 cc. blood containing epinephrine perfused through the cerebral vessels; C, the 125 cc. of the perfused blood injected into the femoral vein.

#### EFFECT ON THE RESPIRATION

It is commonly known that the intravenous injection of epinephrine often causes a disturbance of respiration.

In cases where small doses are injected there may be observed an increase in both the depth and rate of the respiratory movements; on the other hand large doses usually diminish the depth and rate and may even stop respiration for a certain period of time.

This effect so far as I am able to judge from the statements made, where the phenomenon is mentioned, is believed to be due to a direct action upon the respiratory center.

In my experiments I found the effect of the drug very variable although it agrees as a whole with the statements made regarding its action on respiration.

In some instances where the perfusion fluid contained a small amount of the drug there was an increase in both the depth and rate of respiration.

In other cases a like amount of the drug would cause a marked slowing or perhaps it would stop the respiration for a short period.

If the perfusion fluid contained larger amounts of the drug there was apt to be a sudden cessation of respiration. In some instances natural respiration did not return but usually the animals began to breathe again after varying periods of time, but the respiration was quite apt to be more or less irregular. In some of the animals the respiration stopped after the first dose of the drug and artificial respiration was employed to sustain life during the rest of the experiment. It may be stated that in the compilation of the results obtained on heart rate and blood pressure, that due care was exercised in excluding all cases where there was any probability that asphyxia might have been in part responsible for the effect.

#### SUMMARY

1. In summing up the results obtained from the experiments, they tend to show that when epinephrine is perfused through the cerebral circulation, it may in a certain per cent of cases cause a slowing of the heart and that this slowing is at least in part due to a direct stimulation of the vagus center.

2. There is certain evidence which strongly suggests the probability that the drug also stimulates the vasomotor center.

3. The effect on the respiratory center is very variable. There is evidence of both stimulation and depression and neither of these effects appear to be governed by the size of the dose of the drug.



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# THE LIBERATION OF EPINEPHRIN FROM THE ADRENAL GLANDS BY STIMULATION OF THE SPLANCHNIC NERVES AND BY MASSAGE

STUDIED BY MEANS OF THE DENERVATED EYE REACTION

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The observations of a number of investigators have indicated that during electrical stimulation of the splanchnic nerves, epinephrin passes into the circulation by the adrenal veins. These observations may be divided into two groups: (1) Those in which blood has been collected from the adrenal veins of one animal, and tested for epinephrin by its action on the blood pressure when injected into the veins of another animal (Dreyer (1), Tscheboksaroff (2), or by its action on such isolated tissues as intestine or uterus segments (Stewart (3) ). (2) Observations in which the liberation of epinephrin has been deduced from changes in the blood pressure or other reactions in one and the same animal. Thus Joseph and Meltzer (4), found that when one superior cervical ganglion was removed from rabbits, and several days later the peripheral end of the splanchnic nerve stimulated electrically, there resulted in the great majority of cases a variable but unmistakable dilatation of the pupil on the ganglion-free side, while the pupil on the normal side remained practically unchanged. They interpreted this as due to the secretion of the adrenals being thrown into the circulation in sufficient amount to produce a dilatation of the pupil on the ganglion-free side, since it is well known that the pupil on that side is much more sensitive than normal, to the action of adrenalin. (Meltzer and Auer (5) ). While by the first group of methods, results



have been obtained going to show that epinephrin is liberated during stimulation of the splanchnics, and permitting even approximate assay of the quantity so liberated, methods included in the second group present certain advantages for the detailed study of the secretion, and especially for the study of its time relations. The epinephrin liberated passes directly into the circulation of the animal whose reactions are to be tested, under, we must suppose, entirely physiological conditions, without loss and without alteration in its chemical or physical-chemical state. The test is made at once, and can be repeated, and the conditions under which it is made can be varied at will. On the other hand, the very circumstance that the splanchnic nerves are stimulated in the same animal in which the reaction presumed to be due to the liberated epinephrin is observed renders it necessary to exclude the possibility that the reaction is a nervous one due directly to stimulation of the splanchnics. Asher (6) endeavored to do this in the case of the rise in blood pressure following splanchnic stimulation by excising the abdominal viscera, except the adrenals, and then stimulating the splanchnics, with the adrenal veins alternately clamped and open. When they were clamped, he saw no rise of blood pressure on excitation of the nerves. Elliott (7), working with cats, has shown that dilatation of the pupil and retraction of the nictitating membrane on stimulation of the splanchnic succeeds when the adrenals are present, but not when they have been excised. He states, however, that even after removal of the adrenals, stimulation of the splanchnics causes the pupil to dilate slightly, and the nictitating to be withdrawn slowly and with more delay. He suggests that this may be due to "adrenalin liberated from other paranglia or from the actual processes of nervous excitation, or it may be from other metabolites."

The "paradoxical" pupil reaction is obtainable with very small quantities of adrenalin. It involves a definite and limited region, the circulation time from the adrenals to which can be easily estimated, while the arterioles concerned in the rise of blood pressure produced by the epinephrin liberated in response to splanchnic stimulation, cannot be so definitely located. For

these reasons we selected the eye reactions as the criterion of the liberation of epinephrin.

*Technique.* Cats and dogs were employed. The superior cervical ganglion was excised on one side under ether anesthesia from 4, 5 or 6 days to as much as 2 weeks before the experiment on the splanchnics was done. The pupil reaction is well obtained in dogs which are of course especially suitable for observations in which blood is to be collected from the adrenals for further tests, or in which larger amounts of adrenal substance than can be obtained from cats are wanted for determining the epinephrin content of the glands. For the anaesthesia in the actual experiments, urethane was used (about 0.75 gram per kilogram for cats, and 1.5 gram per kilogram for dogs) with ether when needed. In most of the experiments the thorax was opened, and one or both of the sympathetic trunks ligated and cut not far above the diaphragm. In a few experiments one or both splanchnics were exposed for stimulation in the abdomen. For massage of the glands, the abdomen is of course always opened.

The reactions studied were dilatation of the pupil and retraction of the nictitating membrane. Since, for our purpose, it was generally essential to determine with as great exactness as possible the time at which an eye-reaction began, the widening of the aperture was not studied in the great majority of the observations (although its occurrence was verified). The eyelids were purposely held apart in order that the commencement of a further dilatation of the pupil might be seen when it was already dilated to a certain extent. Under the conditions of our experiments, the dilatation of the pupil elicited by splanchnic stimulation began somewhat sooner than retraction of the nictitating. The interval between the commencement of the two reactions varied from 1 to 2 seconds, being influenced to some extent by the dose of epinephrin, and therefore varying somewhat in one and the same animal with the strength and duration of the stimulus.

We began by showing that when the blood from the adrenals is prevented from reaching the eyeball, stimulation of the peripheral end of the splanchnic nerves does not cause the reaction. Excision of the adrenals is of course not a suitable method of excluding them, since it only permits one observation. All that is necessary is to clip the vein from the adrenal whose splanchnic is being stimulated, or if both are being stimulated,

to clamp the inferior vena cava. In a few observations both cava and aorta were clamped. Another simple method is to stop the heart by stimulating the vagus. The eye reaction is never obtained under these conditions, that is to say the pupil of the denervated eye does not dilate in the absence of dilatation of the pupil of the normal eye, nor is the nictitating membrane retracted.

#### EXPERIMENTS IN WHICH THE VENOUS PATH FROM THE ADRENALS WAS OCCLUDED

A certain amount of dilatation may take place simultaneously in both pupils during occlusion of the inferior vena cava, but this is clearly associated with the accompanying asphyxia, and is easily distinguished from the genuine reaction which involves only the denervated eye. When the clamp is removed from the adrenal vein or the cava, the reaction is obtained in the denervated eye and after precisely the same time interval, allowing for the slight variations in successive observations, as is seen when the splanchnics are stimulated without occlusion of the vessels. It is impossible to interpret this result in any other way than that the epinephrin given off in response to the splanchnic stimulation is lying in the adrenal capillaries or veins ready to move out into the circulation at the moment of removal of the clamp.

Thus in an experiment on a cat, the condensed protocol of which is given in table 6 stimulation of the right splanchnic in the thorax was followed by dilatation of the left (denervated) pupil in 9 seconds, and withdrawal of the nictitating membrane. The inferior cava was then clamped above the diaphragm and kept clamped while the splanchnic was again stimulated. No change occurred in the left eye till 9 seconds after removal of the clamp, when dilatation of the pupil began, followed by retraction of the nictitating. The cava was again clamped and the splanchnic stimulated. Dilatation of the pupil occurred 10 seconds after removal of the clamp. Another stimulation of the splanchnic (with the cava free) gave dilatation of the pupil in 8 seconds from the beginning of stimulation. As the cava had been twice clamped for 30 seconds within an interval of 4 minutes, it is pos-



sible that interference with the circulation in the iris, owing to the fall of blood pressure, might have caused the slight lengthening in the time interval (from 9 to 10 seconds) of the reaction obtained after removal of the clamp. In any case, absolute uniformity in the signalling of the moment at which such a reaction is observed to begin cannot be expected, and the closeness of the agreement of successive readings is really remarkable. Later on in this experiment, although numerous injections of methylene blue and of adrenalin solution had been made in the interval, the reaction caused by stimulation of the splanchnic was still obtained 10 seconds after removing the clamp from the cava. At the very end of the experiment, however, when the animal was obviously in bad condition, the pupil reaction did not occur till 19 seconds from the beginning of stimulation of the splanchnic (with vena cava free). In another experiment on a cat, from which the left superior cervical ganglion had been removed 6 days previously, the time interval between the beginning of stimulation of the splanchnic (in the thorax) and the beginning of dilatation of the pupil (followed by retraction of the nictitating) was 9 seconds. Seven successive observations gave 10, 8, 9, 9, 9, 9, 9.2 seconds. The cava was clamped, the splanchnic stimulated and the clamp removed after stimulation had been stopped. The eye-reaction appeared in 9 seconds after removal of the clamp. Later on in the experiment, the time interval between beginning of stimulation of the splanchnic and beginning of the eye-reaction was as usual notably increased (to 12, 15, 15, 19.1, 20, and 13.1 seconds in different observations). In the meantime, repeated injections of methylene blue and adrenalin solution had been made. Atropin solution had also been instilled into the right (normal) eye to enable the circulation time to the retina to be determined.

In an experiment on a cat (whose left superior cervical ganglion had been excised 7 days previously) towards the end of the experiment the time interval of the reaction as elicited by stimulation of the splanchnic (in the thorax) had much increased (from 9.3 seconds at the beginning of the experiment to 15.8 seconds). The right splanchnic was stimulated for a minute during occlusion of the inferior cava. There were deep asphyxial respirations during which both pupils dilated, but contracted again before removal of the clamp. On removing the clamp after an occlusion lasting 63 seconds, the left pupil dilated in 15.2 seconds. Stimulation of the right splanchnic with the cava free was followed by dilatation of the left pupil in 15.8 seconds. Movement of the nictitating membrane was not signalled till 19.2 seconds.



It is particularly important to note that when such an increase in the time interval of the eye reaction elicited by stimulation of the splanchnic with the vessels open has occurred, the same increase is found when the reaction is evoked by releasing the cava when the splanchnic has been excited during its occlusion. The same factors must therefore be responsible in both cases for the greater interval after which the reaction is obtained. Of these factors slowing of the circulation can sometimes be shown to be one. Now slowing of the circulation could only affect the time-interval to exactly the same extent in the two cases if the reaction were caused by a substance moving with the blood. Other factors in the increased time-interval may be increasing sluggishness of response of the mechanisms of the eye and, as will be shown later on, diminished liberation of epinephrin by stimulation of the splanchnics.

#### EXPERIMENTS IN WHICH THE ARTERIAL FLOW TO THE ADRENALS WAS INTERFERED WITH

Some experiments were made with the view of determining whether the reaction could be obtained when the splanchnics were stimulated with the aorta clamped just above the diaphragm. Here, the circulation through the eyeball is of course not interfered with, and if epinephrin in sufficient quantity is liberated into the capillaries of the adrenals, and a sufficient movement of blood in the veins is still present to enable it to arrive at the heart, there is no reason why the usual response should not be elicited, even while the aorta remains occluded. When stimulation of the splanchnic was begun simultaneously with the application of the clamp an eye response was always observed, provided that the splanchnic stimulation was yielding such a response with the circulation free. Accordingly, a brief interference with the arterial flow through the glands does not abolish the secretion. In different experiments, however, there was a certain variability in the time at which the eye reactions appeared. Usually a response was obtained while the aorta still remained obstructed, and a second response after the clamp

was removed. The time interval between the beginning of stimulation and the first response, or between the removal of the clamp and the second response, is of the same order of magnitude as the time interval of the reaction with the vessels free. In other experiments no eye reaction was seen while the clamp remained on the aorta, but a response occurred (with the usual, or a somewhat longer time interval) after removal of the clamp.

Thus, in an experiment on a cat, whose left superior cervical ganglion had been removed 1 week previously, stimulation of the right splanchnic in the thorax was followed by dilatation of the pupil (and withdrawal of the nictitating membrane) of the left eye in 11.2 seconds. Five minutes later the aorta was clamped and the splanchnic stimulated for the same length of time, and with the same distance between the coils as in the previous observation. There was no effect on the eye for the 25 seconds during which the clamp was kept on, but 14 seconds after its removal the pupil dilated, and a little later the nictitating membrane moved back.

In an experiment on a dog, whose left superior cervical ganglion had been removed 6 days previously, the left splanchnic (in the thorax) was stimulated until the pupil of the left (denervated) eye began to dilate in 10.2 seconds from the beginning of stimulation. Two minutes later the left splanchnic was again stimulated, and dilatation of the pupil was obtained in 10.4 seconds. The aorta was then clamped and the splanchnic stimulated for 15 seconds with the same distance between the coils. Dilatation of the pupil occurred at 14.2 seconds from the beginning of stimulation, while the clamp was still on. The clamp was removed after an occlusion of 25 seconds, but no further change was observed in the eye. In another observation in the same animal, a few minutes later, the aorta was clamped and the splanchnic stimulated for 15 seconds. Dilatation of the pupil took place 14.6 seconds from the beginning of stimulation, while the clamp was still on. On removing the clamp, a second dilatation occurred in 9.8 seconds. The first dilatation was slight and remained so till the second carried the response on to moderate dilatation.

In an experiment<sup>1</sup> on a cat, to which morphin had been administered, and whose pupils remained dilated, stimulation of both splanchnics (in the thorax) for 2 seconds caused retraction of the nictitating membrane

<sup>1</sup> The condensed protocol is given in table 8.

and widening of the aperture of the denervated eye in 13 seconds. Two minutes later the observation was repeated, with the aorta clamped, and the eye-reactions appeared in 14.8 seconds, while the clamp was still on. After an interval of 2 minutes, stimulation of the splanchnics was again made for 5 seconds, and the eye response was elicited in 11.2 seconds, with the aorta open. The observation was repeated, the splanchnics being stimulated for the same length of time, with the aorta clamped, and the response occurred in 11.8 seconds, the clamp being still on the aorta. An observation was then made in which the aorta was clamped for 5 seconds before stimulation of the nerves was begun. It remained clamped until the eye response was obtained 11.8 seconds from the beginning of stimulation. On releasing the aorta, further retraction of the nictitating membrane occurred in 15.4 seconds from the moment of removal of the clamp. Forty-eight minutes later, stimulation of the splanchnics for 10 seconds caused a slow retraction of the nictitating in 15 seconds, with the aorta free, and in 15.4 seconds with the aorta clamped.

The explanation of the variability in the results of stimulation of the splanchnics with the aorta clamped is, we suggest, as follows: When the arterial flow is stopped for such periods as we have employed, epinephrin is still liberated into the blood vessels of the adrenals in response to stimulation of the nerves. Blood, doubtless containing some of the epinephrin, since the latent period of the secretion is quite short, continues to move out of the adrenal veins and along the cava after the clamping of the aorta, so long as the pressure remains higher in the arteries peripheral to the clamp than in the veins. As soon as this blood has reached the heart, there is, of course, no reason why it should not be carried as rapidly as before to the eyeball, and no reason why it should not produce there the characteristic reactions, provided that the quantity of epinephrin in it is sufficient. When the clamp is removed from the aorta, a residue of blood containing the balance of the liberated epinephrin is still lying in the adrenal vessels, and begins in its turn to move towards the heart. It is clear that it will depend upon the amount of epinephrin in these two fractions whether a detectable reaction shall be produced in the eye while the clamp is still on the aorta, or



only after its removal, or both after and before removal of the clamp.

The fact that as a rule the time interval of the reaction is somewhat longer when stimulation of the nerves is made with the aorta clamped than when it is made with the vessels free in all probability depends in part on this division of the epinephrin charge liberated by a given stimulation into two parts. For it can be shown that doses of adrenalin which cause, on injection, into a vein, only a minimum eye response, elicit this response after a somewhat greater interval of time than larger doses. Further, the passage of blood from the adrenals to the heart in the first few seconds after clamping the aorta must be slower than with the vessels free, although observations on the injection under minimum pressure of very small volumes of adrenalin solution into the central end of the femoral vein, with the aorta clamped, indicate that this slowing is not important in comparison with the total time interval of the reaction. Some retardation of the response after unclamping the aorta might be expected to be caused by the fact that the depleted vessels have first to be filled up by the arterial blood.

The maximum effect on the eye and the duration of the effect were in general less where the splanchnics were stimulated with the aorta clamped, than in control observations with the vessels free. The division of the liberated epinephrin into two fractions, as suggested, is a sufficient explanation, even if the total amount liberated during a brief interference with the arterial circulation remains undiminished. It is further in accordance with this explanation, that the time interval of the eye response when the splanchnics are stimulated with the inferior vena cava clamped, and the clamp then removed, agrees rather with the time interval of the response elicited with the vessels free than with the aorta clamped. This is also true of the maximum response reached for a given stimulation. Where the vena cava is clamped the epinephrin liberated by the stimulation of the nerves accumulates, of course, behind the clamp, and passes on as a single charge after removal of the clamp.



A period of 5 to 10 seconds of anemia (caused by clamping the aorta) preceding stimulation of the splanchnics, had no noticeable effect in diminishing the reaction. When the preliminary period of anemia was increased to 20 seconds, in the same animal (see table 8), stimulation of the nerves was without result in one observation, but in another observation an eye response was obtained when the splanchnics were excited after a preliminary anemia of 20 seconds. We have not hitherto made any experiments with the view of determining the precise period of anemia which will prevent a reaction. It is to be expected, of course, that it will vary to some extent in different animals, and at different times in an experiment on the same animal.

That epinephrin is actually carried in the blood of the inferior cava to the heart, while the aorta remains occluded, can be shown by injecting adrenalin solution into the central end of the femoral vein under the minimum pressure necessary to permit it to enter the vein, and in such small volume that it cannot possibly be mechanically forced along to the heart during the injection. The eye reactions are obtained after a time interval of the same order of magnitude, whether given quantities of adrenalin solution are injected with the aorta free or clamped. The interval may, indeed, be shorter with the aorta clamped. This is precisely the case when a given dose of adrenalin is injected in the form of a smaller volume of stronger solution than in the form of a larger volume of weaker solution, as is well illustrated in the first part of the protocol reproduced in table 8.

Thus, 0.02 mg. adrenalin injected (between 3.15 and 3.42 p.m.), in the form of 2 cc. of a 1: 100,000 solution, caused an eye-reaction at very much the same time interval as 0.02 mg. injected in the form of 0.5 cc. of 1: 25,000 adrenalin, with the aorta free (average time for the weaker solution 9.2 seconds; for the stronger 8.4 seconds). With the aorta clamped, the same quantity of the weaker solution gave a time of 10.8 seconds, and the stronger an average time of 7.8 seconds. While there are, of course, variable factors in such observations, which cannot all be controlled, the difference is suggestive. Later on in the experiment, when the time interval of the reaction elicited by stimulation of the splanchnic (with cava clamped) had considerably increased (to 16.6

seconds at 6.00 p.m.), the difference was even more striking. 0.1 cc. of a 1: 25,000 solution of adrenalin injected with the aorta free, gave the response after an interval of 16.6 seconds (at 6.01 p.m.), the retraction of the nictitating membrane being very slow, but steadily progressing. Later on 0.2 cc. of the same solution gave a good response after an interval of 16.8 seconds (at 6.36 p.m.), while 0.2 cc. of the solution, injected with the aorta clamped, gave an interval of 11.6 seconds, and 0.1 cc. an interval of 9.8 seconds. The injection of 0.04 cc. of 1: 25,000 adrenalin (with the vessels open), was followed by very slow retraction of the nictitating in 17 seconds.

These results are puzzling at first sight, but in all probability they depend upon two factors. First, the clamping of the aorta increases the blood pressure, and diminishes the circulation time in the head end of the animal. This will be especially influential in diminishing the interval at which the eye response to adrenalin injection occurs when the circulation has already deteriorated, as towards the end of a long experiment. Among other things, the action of the heart will be improved by the better filling of the coronary vessels, and the latency of the structures in the eye-ball may also be diminished by the improved circulation. Provided, then, that the adrenalin reaches the heart without undue delay, we should expect the response to occur sooner with the aorta clamped. But why should injection of a larger volume of weaker solution (with aorta clamped) not evoke the response in the same time, as the injection of a correspondingly smaller volume of the stronger solution? If the volume of the weaker solution were so great that practically the whole of it passed directly under the injection pressure into the heart, there could, of course, be no such delay as was observed. But the residual movement in the inferior cava after the aorta has been clamped may not be adequate to rapidly transport such a volume as 2 cc., while the whole or the greater part of such a volume of adrenalin solution as 0.1 to 0.5 cc. may be quickly transported to the heart. The eye reaction elicited when the greater volume was injected would be a reaction corresponding to a smaller concentration and to a smaller total quantity, than when a smaller volume of the stronger solution was injected.

EXPERIMENTS IN WHICH THE SPLANCHNICS WERE STIMULATED,  
WHILE THE CIRCULATION WAS STOPPED OR SLOWED BY  
INHIBITION OF THE HEART

Further evidence that the eye reaction is entirely due to a substance (epinephrin) moving in the blood from the adrenals is obtained by stopping the circulation by stimulation of the vagus.

In an experiment on a dog, the eye reaction was got in 10.2 seconds from the beginning of stimulation of the left splanchnic in the thorax. In another observation immediately thereafter, the time was 10.4 seconds. The peripheral end of the left vagus was now stimulated so as to cause complete disappearance of the pulse in the aorta. Three seconds later the left splanchnic was stimulated. Excitation of the vagus was continued for 15 seconds. The (denervated) eye reaction did not appear till 20 seconds from the beginning of stimulation of the splanchnic, or 8 seconds after stoppage of the vagus stimulation. Four minutes later the splanchnic was again stimulated, simultaneously with the vagus, the stimulation of the latter continuing for 35 seconds. No eye-reaction appeared during all this time. Three minutes thereafter stimulation of the splanchnic alone gave an eye reaction in 8.2 seconds. The vagus was again stimulated for 33 seconds so as to stop the pulse in the aorta. The splanchnic was stimulated for 15 seconds with a stronger current than in the previous observations. Eye reactions were obtained 42 seconds after the beginning of the stimulation of the splanchnic, or 11 seconds after cessation of vagus stimulation. Stimulation of the splanchnic alone now gave an eye reaction in 8.4 seconds.

Fully as convincing as the experiments in which complete stoppage of the circulation was produced, are those in which it was simply slowed by the vagus or by hemorrhage.

In an experiment on a cat (whose left superior cervical ganglion had been removed a week before), stimulation of the right splanchnic in the thorax was followed by the eye reaction in 10 seconds from the beginning of stimulation. Stimulation of the peripheral end of the right vagus and of the splanchnic was then made simultaneously. The heart was slowed but not stopped. The eye-reaction appeared in 15.2 seconds. The splanchnic was again stimulated alone, and the eye-reaction occurred in 10.2 seconds. Another observation was thereafter made with simultaneous stimulation of the vagus and splanchnic.



The eye reactions (pupil dilatation and retraction of nictitating) appeared in 15.2 seconds. When the splanchnic only was now stimulated, the reaction occurred in 11.2 seconds. Another vagus and splanchnic observation in which stimulation of the vagus was begun in advance of the splanchnic stimulation, so as to be sure that the slowing of the circulation embraced the whole time of passage of the epinephrin to the eyeball, gave the eye reactions after an interval of 15 seconds from the beginning of excitation of the splanchnic.

Later on in the same experiment the eye reaction was obtained 15.8 seconds after the commencement of stimulation of the splanchnic. Hemorrhage was now produced from the femoral artery, and the splanchnic stimulated immediately thereafter. The eye reaction did not occur till 21.6 seconds from the beginning of stimulation.

#### COMPARISON OF EYE REACTION ELICITED BY INJECTION OF ADRENALIN AND BY STIMULATION OF THE SPLANCHNICS. LATENT PERIOD OF EPINEPHRIN SECRETION

Another method of showing that the eye reactions studied are caused by epinephrin liberated from the adrenals, is to inject a small amount of adrenalin into the left renal vein, or, what comes almost to the same thing for this purpose, into the central end of a femoral vein; and to determine the time from the beginning of injection to the beginning of the eye reaction. The femoral vein has the advantage over the renal that its use does not entail opening of the abdomen, or manipulation in the neighborhood of the adrenals. The circulation time from the mouth of the femoral vein, up to which the column of adrenalin solution extends before injection, will of course be somewhat greater than that from the adrenal veins. But for our purpose the difference is not important, as shown by circulation time measurements.

In an experiment, on a cat, the following results were obtained: Stimulation of the left splanchnic in the thorax gave in three successive observations, 9.4, 11.2 and 10.8 seconds as the time interval of the eye reaction. Stimulation of the right splanchnic gave 11.8 seconds, of both splanchnics together in two observations, 10.6 and 10 seconds, respectively. Injection of 1 cc. of 1:25,000 adrenalin solution in Ringer's, into the central end of the femoral vein was followed by dilatation of the



pupil in 10.6 seconds. The same amount of adrenalin was now injected while the vena cava was clamped. The pupil was unaffected till 11.2 seconds after removal of the clamp, when it dilated. Both splanchnics were now stimulated, and the eye reaction was obtained in 11.4 seconds. Injection of 0.5 cc. of 1:25,000 adrenalin into the femoral vein was followed by dilatation of the pupil in 11.2 seconds.

The quantities of adrenalin injected in this experiment were such as caused about the same reaction in the eye as the stimulation employed for the splanchnics. It is clear enough that with such quantities, the interval between the beginning of injection and the beginning of the eye reaction is approximately the same whether the reaction is elicited by adrenalin artificially introduced, or by epinephrin liberated from the glands. This indicates that the epinephrin liberated by splanchnic stimulation passes so rapidly into the veins that the latent period does not constitute a fraction of the total time interval to the commencement of the eye reaction long enough to be appreciated in such measurements. So far as these observations go, the indication is also that the material as liberated from the adrenals is, as regards the production of the reaction studied, the same as adrenalin.

On the other hand, if the quantity of adrenalin injected is much greater than is required to produce an effect upon the eye equal to that caused by splanchnic stimulation, the time interval between the beginning of the injection and the beginning of the eye reaction is reduced decidedly below that between the beginning of stimulation of the splanchnic and the commencement of the eye reaction, although it always remains, of course, greater than the circulation time to the carotid.

This is illustrated in the following experiment on a cat: 2 cc. of adrenalin (1:50,000) in methylene blue solution, was injected into the femoral vein. The methylene blue first reached the carotid in 3 seconds, and the pupil began to dilate in 5.2 seconds. In another observation, 5 minutes later, the numbers came out 3.2 seconds for the circulation time to the carotid, and 5.6 seconds for the pupil reaction. Before the first adrenalin injection, the time interval for the pupil reaction elicited by stimulation of the splanchnic was 9 seconds

in one observation, and 8 seconds in another. In an observation made 5 minutes after the second injection of adrenalin, the time interval of the eye response following splanchnic stimulation, was 8.5 seconds. The reduction of the interval, in the case of the adrenalin injection, could not be due to a change in the circulation time. It must be attributed to a reduction in the latent time of the eye mechanisms. It is probable that the time required for the blood to pass from the carotid through the capillaries of the iris to the veins, would be not much less than 2 seconds. Accordingly in this strength (and with an iris as sensitive as in this animal in the earlier part of the experiment) the adrenalin began to produce its effect, upon the pupil very soon after the first blood containing it had completely traversed the sensitive structures. Later on in the same experiment, the time interval both for splanchnic stimulation and for injection of adrenalin increased, while the circulation time to the carotid remained the same (3 seconds). This also can only be due to increasing sluggishness of the iris, with perhaps some increasing vasoconstriction in peripheral vessels including those of the eyeball.

Of course, by forcibly injecting the adrenalin solution under considerable pressure, it is possible to produce a definite diminution in the time interval of the eye response, since under these conditions, the passage of the adrenalin from the point of injection to the heart is practically instantaneous, and the pressure in the right heart may also be artificially raised, and the circulation time through the lungs correspondingly shortened. This is well illustrated in table 7, in the observations at 5.48 and 5.58 p.m., and in those between 7.12 and 7.18 p.m.

In another experiment, on a dog, the results shown in table 1 also illustrate the fact that with different doses of adrenalin different time intervals may be obtained for the (denervated) eye reaction.

In this dog (weighing 5.5 kg.), the superior cervical ganglion had been removed 6 days before the experiment. The eye-reactions were elicited with great ease by stimulation of the splanchnics and by massage of the glands; and also by very small doses of adrenalin. Exactly the opposite was the case with the cat some results from which are given in table 2. Here the eye reactions were obtained with much greater difficulty through

splanchnic stimulation, and relatively large doses of adrenalin were required to bring them out. When stimulation of the splanchnics finally became ineffective, massage of the glands yielded no result, or only a doubtful one. The epinephrin content of the glands, removed in both animals after a long experiment, was in this dog the highest which we have hitherto observed in the course of the work, while in the cat it was with one exception the lowest. The cat had brought forth a litter of kittens 13 days before the experiment, and was suckling them, and the adrenals were enlarged. It is not known whether the low epinephrin content was correlated with the parturition and lactation. The left adrenal of the cat weighed 0.317 gram, and contained 0.12 mg. epinephrin. The right adrenal weighed 0.301 gram, and also contained 0.12 mg. epinephrin.

TABLE 1

TIME	INJECTED INTO FEMORAL VEIN	PUPIL DILATATION BEGINS IN
		<i>seconds</i>
2.15	1.0 cc. Adrenalin (1: 50,000).....	10.8
2.17	0.5 cc. Adrenalin (1: 50,000).....	11.4 (less dilatation)
2.22	0.4 cc. Adrenalin (1: 50,000).....	13.0 (small effect)
2.26	2.0 cc. Adrenalin (1: 250,000).....	12.4
2.28	1.0 cc. Adrenalin (1: 250,000).....	11.2
2.30	0.5 cc. Adrenalin (1: 250,000).....	16.6

It must be stated that our estimations were almost invariably made on the adrenals of animals on which more or less prolonged experiments had been carried out. High epinephrin contents were therefore not to be expected.

The increasing time interval of the response with stimulation of the splanchnics, in the latter part of this experiment (table 2), has the same significance as increase of the time interval with small doses of adrenalin. For the pupil dilatation obtained, from successive stimulations of the nerves, became less and less, and, as already stated, when the maximum dilatation and the duration of a reaction are small, the time of onset of the reaction is delayed. Another point well brought out in this experiment was that doses of adrenalin, which, at one stage are ineffective, may



TABLE 2

*Condensed Protocol of experiment on a cat, weighing 2 kg., whose left superior cervical ganglion had been removed 15 days before the experiment*

TIME		PUPIL DILATATION BEGINS IN
		<i>seconds</i>
12.18	Stimulated both splanchnics for 10 seconds.....	11.4
12.31	3 cc. Adrenalin (1: 25,000).....	9.8
12.36	1 cc. Adrenalin (1: 25,000).....	11.2
12.43	1 cc. Adrenalin (1: 100,000).....	17.8 Dilatation very slight
12.45	3 cc. Adrenalin (1: 100,000).....	11.8 About same dilatation as with splanchnic stimula- tion
12.48	1 cc. Adrenalin (1: 25,000).....	12.8
12.50	1 cc. Adrenalin (1: 25,000).....	12.2
12.52	2 cc. Adrenalin (1: 25,000).....	12.4 Better dilata- tion than with 1 cc.
12.58	2 cc. Adrenalin (1: 25,000) (with cava clamped). Cava released.....	No dilatation 13.0
1.00	Both splanchnics stimulated 10 seconds.....	13.6 Dilatation small
1.03	2 cc. Adrenalin (1: 25,000).....	11.0
1.06	2 cc. Adrenalin (1: 25,000) (with cava clamped). Cava released.....	No dilatation 12.0
1.08	Both splanchnics stimulated for 10 seconds.....	15.0 Slight dilata- tion
1.27	2 cc. Adrenalin (1: 40,000).....	11.0 Dilatation prolonged
1.29	1 cc. Adrenalin (1: 40,000).....	13.8
1.30	1.4 cc. Adrenalin (1: 40,000) (with cava clamped)..... Cava released.....	No dilatation 12.0
1.39	1 cc. Adrenalin (1: 40,000) (with cava clamped). Cava released.....	No dilatation 13.2
1.58	2 cc. Adrenalin (1: 40,000) (with aorta clamped for 14 seconds from beginning of injection)..	13.2
2.00	Both splanchnics stimulated.....	No dilatation



later on become effective; and still later may again cease to yield a response.

Thus, between 11.58 and 12.06, four observations (not reproduced in the Table) were made, in which were injected respectively 1 cc. of adrenalin (1:100,000), 2 cc. of adrenalin (1:100,000), 2 cc. of adrenalin (1:50,000) and 3 cc. of the 1:50,000 solution without effect on the eye. Between 1.16 and 1.19 p.m., three injections, two respectively of 2 cc., and one of 3 cc. of 1:100,000 adrenalin were without result. As shown in table 2, at 12.43 p.m., 1 cc. of the same solution, and at 12.45 3 cc., gave positive results. Naturally, variations in the anesthesia may play a part, in this variability of the response, and the point is mentioned merely to illustrate the fact that numerous controls are required in such observations.

It has been already stated that in prolonged experiments, the increase in the time interval between the beginning of stimulation of the splanchnics, and the beginning of the eye reaction, which is observed towards the end of the experiment, may be shown by measurements of the circulation time to be partly due to slowing of the circulation. Another factor, however, is involved, namely, the increase in the latency of the iris response, after the epinephrin has reached the eye. In the time interval, three factors are plainly concerned: first, the latent period of the adrenal gland, that is, the time that elapses between the beginning of an effective stimulation and the appearance of epinephrin in the blood; (2) the circulation time from the adrenal to the eye ball; (3) the latent period of the structures in the eye ball which yield the reaction. The observations already mentioned show that the first factor constitutes a very small part of the whole time interval. It may vary with the strength of the stimulus to some extent, but this cannot easily be determined, because the moment at which the eye reaction can just be seen to begin also depends somewhat on the amount of epinephrin liberated. In comparison with the time required for its transportation to the eye, and with the lost time there before the sensitive structures respond, the latent period of the secretion may be considered negligibly small.

MINIMUM DURATION OF EFFECTIVE STIMULATION OF THE  
SPLANCHNICS

The minimum time of stimulation necessary to produce the liberation of an amount of epinephrin just sufficient to cause an appreciable reaction, is also very short. It varies, of course, with the strength of stimulation. With a stimulus (faradic) strong enough to be sharply felt on the tongue, one-half of a second of stimulation sufficed to give a reaction. With increase in the time of stimulation, the maximum amount of dilatation of the pupil obtained and the duration of the dilatation increased, and retraction of the nictitating membrane, which was not got with the short periods of stimulation, was seen. The maximum effect in both reactions was reached more speedily with increas-

TABLE 3

TIME	DURATION OF STIMULUS	BEGINNING OF PUPIL DILATATION	NICTITATING RETRACTED
	<i>seconds</i>	<i>seconds</i>	
10.44	1	8.0	+
10.45	$\frac{1}{2}$	9.0 (slight)	-
10.47	3	9.0	+
10.48	5	9.0	+
10.50	10	9.0	+
10.52	15	9.2	+

ing time of stimulation. In an animal in which a reaction is being easily elicited the total time interval, between the beginning of stimulation and the commencement of the eye reaction, may be approximately the same for the longer as for the shorter periods of stimulation. This is well shown in tables 3 and 4, taken from experiments on two cats. Where the eye reaction (or the adrenal secretion) is becoming exhausted in the course of a long experiment the minimum time of stimulation needed to produce an effect may be greater than earlier in the experiment. When this is the case, the time interval to the beginning of the eye reaction may be diminished as the duration of the stimulus is increased, as is shown in table 5, taken from the same experiment as table 4.

COMPARISON OF CIRCULATION TIME FROM ADRENALS TO EYEBALL,  
WITH TIME AT WHICH THE EYE REACTION APPEARS

The circulation time from the level of the adrenals to the carotid and to the eyeball was determined in a number of experiments by the methylene blue method (8), in order to verify the assumption that the time interval of the eye reaction was of the order of magnitude appropriate to a reaction elicited by a substance moving from the adrenals to the eye with the average speed of the blood. In every case the time required for blood to

TABLE 4

TIME	DURATION OF STIMULUS	BEGINNING OF PUPIL DILATATION	NICTITATING RETRACTED
	<i>seconds</i>	<i>seconds</i>	
11.32	5	9.6	+
11.35	$\frac{1}{3}$	9.6 (very slight)	—
11.37	1	10.2	—
11.38	3	10.0	in 11 seconds

TABLE 5

TIME	DURATION OF STIMULUS	BEGINNING OF PUPIL DILATATION	NICTITATING RETRACTED
	<i>seconds</i>	<i>seconds</i>	
12.35	15	13.4	13.4
12.38	$\frac{1}{3}$	No dilatation	—
12.40	1	No dilatation	—
12.42	3	No dilatation	—
12.44	5	18.6 (very slight)	—
12.46	10	15.0 (very slight)	—

pass to the eye or even through it, was found to be less than the time interval at which the reaction occurred when elicited by stimulation of the splanchnics. A margin was always left for the time necessary for the reaction to be developed to the point at which it could be detected after the epinephrin had reached the sensitive structures.

In an experiment, on a cat, 1.5 cc. of a solution of methylene blue made with Ringer's solution, was injected into the left renal vein.

The right splanchnic in the thorax was stimulated from the beginning

of the injection, the stimulation being continued for 5 seconds. The methylene blue was first observed in the carotid at 4.2 seconds, and the pupil began to dilate at 10.2 seconds from the beginning of injection. 1.5 cc. of 1:50,000 adrenalin in the same solution of methylene blue was then injected into the femoral vein. The color was seen in the carotid at 6 seconds, and dilatation of the pupil occurred at 10.2 seconds. An injection of 2 cc. of the adrenalin-methylene blue solution was then made, and the color was observed in the carotid at 5.2 seconds, the dilatation of the pupil beginning at 9.2 seconds. Later on in the experiment, when the animal was in distinctly worse condition, stimulation of the right splanchnic (for 15 seconds) gave dilatation of the pupil and retraction of the nictitating on the denervated side in 13.2 seconds from the beginning of stimulation. Injection was then made into a femoral vein of 2 cc. of 1:50,000 adrenalin in methylene blue solution. Dilatation of the pupil occurred in 12.2 seconds. In a later observation 4 cc. of the adrenalin methylene blue solution was injected. The blue was seen with the ophthalmoscope in the retinal vessels of the normal eye at 11.4 seconds, and the pupil dilatation occurred in the denervated eye at 14.4 seconds. The normal eye was dilated with atropia. When this observation was repeated, 7 minutes later, the color was detected in the retina in 10.6 seconds, and dilatation of the pupil took place in 12.2 seconds.

The protocol in table 6 further illustrates experiments in which simultaneous observations of the eye reaction caused by adrenalin, and the circulation time, as determined by the methylene blue method, were made.

The circulation time from the left renal vein or the femoral vein to the carotid artery corresponds to a little less than the time required for the first of the epinephrin liberated from the adrenal to reach the iris. Probably something like 2 seconds must be added for the circulation time through the iris capillaries. (It was found by one of us (9) that the time from the central artery to the central vein of the retina in chloralized rabbits was from 1.7 to 1.9 seconds.) Even allowing for this, it is evident that it takes some sensible time after epinephrin has traversed the sensitive structures in the eyeball before a reaction is seen, at least under the conditions of our experiments.



TABLE 6

*Condensed protocol of experiment on a cat. Left superior cervical ganglion removed  
16 days before the experiment*

TIME		SEEN IN CAROTID AFTER	PUPIL REACTION
		<i>seconds</i>	<i>seconds</i>
10.20	Urethane 1 gram.....		
11.00	Right splanchnic ligated and cut in thorax..		
11.02	Stimulated splanchnic.....		9.0
11.04	Inferior vena cava clamped; splanchnic stimulated for 15 seconds. No effect on the pupils while the cava was clamped; after 30 seconds cava unclamped, and 9 seconds later pupil dilated.....		9.0
11.08	Repeated observation made at 11.04.....		10.0
11.10	Stimulated splanchnic.....		8.0
11.40	Injected 2 cc. methylene blue into renal vein.	4.0	
11.42	Injected 1.5 cc. methylene blue into renal vein.....	3.8	
11.44	Injected 1.5 cc. methylene blue into renal vein.....	3.0	
11.46	Injected 1.5 cc. " " " "	4.0	
11.48	Injected 1.5 cc. " " " "	3.2	
11.59	Injected 2 cc. of 1:50,000 adrenalin in methylen blue.....	3.0	5.2
12.02	Repeated last observation.....	3.2	5.6
12.07	Stimulated splanchnic.....		8.5
12.10	Clamped cava for 32 seconds. Stimulated splanchnic 15 seconds from the time of clamping.....		No dilatation
	Removed clamp.....		10.0
12.15	Clamped cava. Injected 2 cc. adrenalin- methylen blue into renal vein.....		No dilatation
	Removed clamp after 24 seconds.....		7.0
12.20	Repeated last observation. Cava clamped 37 seconds.....		No dilatation
	Removed clamp. Methylene blue seen in carotid in.....	3.0	9.5
12.25	Injected 2 cc. adrenalin-methylen blue into renal vein.....	3.3	8.2
12.40	Stimulated splanchnic for 15 seconds.....		19.0
12.42	Stimulated splanchnic with stronger stimulus.		No effect
12.56	Massaged left adrenal.....		No effect

EXPERIMENTS IN WHICH THE ARTERIAL FLOW TO THE EYEBALL  
WAS INTERFERED WITH

The experiments on occlusion of the venous path from the adrenals were supplemented by observations in which the arterial path to the eyeball was interfered with. The circulation in an eyeball is not completely stopped when the corresponding common carotid is occluded. If, then, the response to stimulation of the peripheral end of the splanchnic is solely due to a substance carried in the blood, it is obvious that it will depend upon the amount of blood reaching the eye by other routes, upon the amount of epinephrin liberated by a given stimulation of the splanchnics and upon the sensitiveness of the reacting structures to epinephrin, whether the reaction yielded by splanchnic stimulation shall be entirely suppressed or merely postponed, and perhaps weakened, by occlusion of one or of both carotids. It ought, indeed, to be merely a matter of properly choosing the duration and strength of stimulation, and the degree of interference with the arterial path to the eye, to bring about complete suppression of the eye reaction or to permit it to be elicited. Further, the details of the eye response to splanchnic stimulation, when the flow of blood to the eye is more or less interrupted, should be capable of being imitated by injecting greater or smaller quantities of adrenalin into a vein. This is fully as severe a test as the occlusion of the venous path from the adrenals. For a greater variety of result is possible. Yet all the variations must agree with the assumption that the effective factor, in bringing about the eye response, is circulating epinephrin. If, for instance, it were found that with one or both carotids clamped the eye reaction followed sooner on stimulation of the splanchnic than with the vessels free, this could not be reconciled with the assumption aforesaid, for it must take longer for the epinephrin to reach the eye ball by less direct routes, and less of it will arrive at the sensitive structures.

As will be seen from the condensed protocol of the experiment reproduced in table 7, the results of clamping the ipsilateral or the contralateral carotid, or of both, with varying intensity and

duration of stimulation of the splanchnic, are invariably what they ought to be if the eye reaction is due to a substance carried in the blood. Also, each result can be imitated by injecting different quantities of adrenalin solution into the femoral vein with one or both carotids clamped. The occlusion of the carotid has the slight disadvantage from the point of view of technique, that it causes some dilatation of the pupil itself. This, however, when the splanchnic stimulation or adrenalin injection is made immediately after clamping, is not a serious drawback, and the beginning of the more sudden dilatation of the pupil due to epinephrin is easily recognized when superposed upon the slow dilatation due to interference with the circulation.

It will be seen that in this animal, in which the eye response was elicited by stimulation of the splanchnic with great ease, it was necessary to reduce the time of excitation even when only one splanchnic was stimulated to 2 seconds, and to reduce the strength of the stimulus in order to abolish the reaction completely when both carotids were clamped. On releasing the carotids, a response was not in general obtained comparable to the response observed when the vena cava is released after a period of stimulation. It is clear that this ought to be the case, because the epinephrin instead of being dammed back behind the clamp, as when the cava is occluded, must have passed for the most part into the general circulation during occlusion of the carotid. It is of course possible that when unusually large quantities of epinephrin are liberated, an after response may be obtained on releasing the carotids. With a shorter distance between the coils, and the same time of stimulation, or with a longer time of stimulation and the same distance between the coils, so much more epinephrin was liberated from the adrenals that enough of it found its way to the denervated eye, even with both carotids clamped, to produce after a much lengthened time interval the characteristic reaction. When only one carotid was clamped, occlusion of the ipsilateral artery had a much greater effect than clamping of the contralateral artery, in lengthening the time interval. The fact that doses of adrenalin could be determined which, when injected into a vein, behaved as

TABLE 7

*Condensed protocol of experiment on a cat weighing 3 kg., whose left superior cervical ganglion had been excised 9 days before. The numbers in brackets are the distances between the coils in stimulating.*

TIME		DILATATION OF PUPIL AFTER	NICTITAT- ING MEMBRANE
		<i>seconds</i>	
1.00	2.5 grams urethane.....		
1.30	Ether.....		
2.15	Left splanchnic ligated, divided and isolated in thorax.....		
2.30	Left splanchnic (stimulated) 9.8 seconds (coils at 12 cm.).....	9.8	+
2.33	Left splanchnic (stimulated) 10 seconds (coils at 12 cm.) (with left carotid clamped).....	12.8	+
2.35	Left carotid clamped for 10 seconds (no effect on eye).....		
2.39	Left splanchnic stimulated for 10 seconds (8.5 cm.).....	10.2	+
2.41	Left splanchnic stimulated for 6.8 seconds (8.5 cm.).....	6.8	+
2.43	Left splanchnic stimulated for 5 seconds (9 cm.).....	7.0	+
2.45	Left splanchnic stimulated for 5 seconds (9 cm.) (left carotid clamped).....	8.0	
2.48	Left splanchnic stimulated for 5 seconds (10 cm.).....	8.6	
2.49	Left splanchnic stimulated for 5 seconds (12 cm.).....	6.8 (moderate)	
2.51	Left splanchnic stimulated for 2 seconds (12 cm.).....	7.2 (moderate)	+
3.02	Left splanchnic stimulated for 1 second (12 cm.) (left carotid clamped).....	10.8 (moderate)	+
3.05	Left splanchnic stimulated for 2 seconds (12 cm.) (both carotids clamped).....	No eff. at 20	-
3.08	Left splanchnic stimulated for 5 seconds (12 cm.).....	7.2	+
3.10	Left splanchnic stimulated for 5 seconds (12 cm.) (both carotids clamped).....	12.4	+
3.14	Left splanchnic stimulated for 5 seconds (12 cm.).....	7.2	+
3.16	Left splanchnic stimulated for 2 seconds (12 cm.).....	10.2	+
3.23	Left splanchnic stimulated for 2 seconds (12 cm.) (both carotids clamped).....	No react. in 25	



TABLE 7—Continued

TIME		DILATATION OF PUPIL AFTER	NICTITAT- ING MEMBRANE
		<i>seconds</i>	
3.26	Left splanchnic stimulated for 2 seconds (12 cm.) (left carotid clamped).....	16.2 (moderate)	—
3.30	Left splanchnic stimulated for 2 seconds (12 cm.).....	7.8	+
3.32	Left splanchnic stimulated for 2 seconds (12 cm.) (both carotids clamped).....	No react. in 30*	
3.35	Clamped both carotids for 25 seconds†...		
3.44	Left splanchnic stimulated for 10 seconds (12 cm.) (both carotids clamped)††.....	12.4	+
3.49	Left splanchnic stimulated for 8.6 seconds (12 cm.).....	8.6	+
3.51	Left splanchnic stimulated for 10 seconds (12 cm.) (left carotid clamped).....	10.2	+
3.55	Left splanchnic stimulated for 10 seconds (12 cm.) (both carotids clamped).....	15.6	+
	No reaction on releasing		
4.00	Left splanchnic stimulated for 5 seconds (12 cm.) (both carotids clamped).....	15.8 (moderate)	—
4.03	Left splanchnic stimulated for 5 seconds (12 cm.) (left carotid clamped).....	No effect	
4.06	Left splanchnic stimulated for 5 seconds (12 cm.).....	8.4	+
4.08	Left splanchnic stimulated for 5 seconds (12 cm.) (left carotid clamped).....	11.0	+
4.09	Left splanchnic stimulated for 5 seconds (12 cm.) (right carotid clamped).....	9.2	+
4.12	Left splanchnic stimulated for 2 seconds (12 cm.).....	7.6	+
4.16	Left splanchnic stimulated for 2 seconds (12 c.m.) (both carotids clamped).....	No effect	
4.19	Left splanchnic stimulated for 2 seconds (12 cm.) (left carotid clamped).....	13.8 (moderate)	+
4.27	Left splanchnic stimulated for 2 seconds (12 cm.) (right carotid clamped).....	9.4	
4.47	1 cc. (1:100,000) adrenalin into femoral vein.....	10.6	
5.07	2 cc. (1:25,000) adrenalin into femoral vein.....	7.8	+
5.12	1 cc. (1:12,500) adrenalin into femoral vein.....	7.4	+
5.14	1 cc. (1:12,500) adrenalin (both carotids clamped).....	11.2	+

TABLE 7—Continued

TIME		DILATATION OF PUPIL AFTER	NICTITAT- ING MEMBRANE
		<i>seconds</i>	
5.15	0.5 cc. (1:12,500) adrenalin (both carotids clamped).....	16.6	+
5.21	0.3 cc. (1:12,500) adrenalin.....	12.8	+
5.25	0.3 cc. (1:12,500) adrenalin (both carotids clamped).....	20.0	+
5.30	0.5 cc. (1:25,000) adrenalin (both carotids clamped).....	17.2	+
5.35	Left splanchnic stimulated for 2 seconds (12 cm.).....	6.0	+
5.37	Left splanchnic stimulated for 2 seconds (12 cm.).....	6.0	+
5.40	Left splanchnic stimulated for 5 seconds (12 cm.).....	6.0	+
5.43	Left splanchnic stimulated for 5 seconds (12 cm.) (both carotids clamped).....	15.0	+
5.45	Left splanchnic stimulated for 2 seconds (12 cm.) (both carotids clamped).....	No reaction	
5.48	0.7 cc. (1:25,000) adrenalin (slowly injected).....	11.2	+
5.58	0.6 cc. (1:25,000) adrenalin (injected faster).....	8.6	+
6.00	1.2 cc. (1:25,000) adrenalin (injected faster).....	6.0	+
6.02	1.2 cc. (1:25,000) adrenalin (both carotids clamped).....	14.2 (moderate)	+
6.04	0.25 cc. (1:25,000) adrenalin (both carotids clamped).....	No reaction	
6.20	Left splanchnic stimulated for 6.2 seconds (12 cm.).....	6.2	+
6.48	0.3 cc. (1:25,000) adrenalin.....	12.0	+
6.50	0.25 cc. (1:25,000) adrenalin.....	12.0	+
6.53	0.3 cc. (1:25,000) adrenalin (both carotids clamped).....	No reaction	
6.54	0.25 cc. (1:25,000) adrenalin (left carotid clamped).....	15.2	+
6.55	0.3 cc. (1:25,000) adrenalin (right carotid-clamped).....	11.6	+
6.56	0.15 cc. (1:25,000) adrenalin (left carotid clamped).....	17.0 (moderate)	+
6.58	0.15 cc. (1:25,000) adrenalin.....	13.4	+
7.00	0.15 cc. (1:25,000) adrenalin (both carotids clamped).....	No reaction	

TABLE 7—Continued

TIME		DILATATION OF PUPIL AFTER	NICTITAT- ING MEMBRANE
		<i>seconds</i>	
7.02	0.15 cc. (1:25,000) adrenalin.....	10.2	+
7.03	Right splanchnic divided isolated.....		
7.06	Right splanchnic stimulated for 8.2 sec- onds (9 cm.).....	8.2	+
7.07	Right splanchnic stimulated for 8.2 sec- onds (9 cm.).....	9.0	+
7.09	Right splanchnic stimulated for 10 sec- onds (12 cm.).....	13.0	+
7.10	Right splanchnic stimulated for 5 sec- onds (12 cm.).....	12.0 (moderate)	+
7.11	Right splanchnic stimulated for 5 sec- onds (9 cm.).....	10.0 (moderate)	+
7.12	0.8 cc. (1:25,000) adrenalin (injected slowly).....	11.0	+
7.13	1.0 cc. (1:25,000) adrenalin (injected slowly).....	11.0	+
7.15	1.0 cc. (1:25,000) adrenalin (injected rapidly under pressure).....	8.4	+
7.18	2.0 cc. (1:25,000) adrenalin (injected rapidly under pressure).....	7.4	+

Left adrenal weighed 0.214 gm. and contained 0.12 mg. epinephrin.

Right adrenal weighed 0.203 gm. and contained a trace of epinephrin (less than 0.02 mg.).

\* When carotids were clamped, gradual dilatation of both pupils occurred, the left going on to almost full dilatation. On removing clamps, left pupil kept on dilating to full, and the nictitating membrane retracted at 12.4 seconds after release of carotids.

† Both pupils gradually dilated, left more than right. After release contraction of both pupils, right contracting immediately and left gradually. The left nictitating retracted 16.4 seconds after release.

†† Removed clamp after 35 seconds. The same effects were observed as is in observation of 3.35, nictating retracting 14.1 seconds after release. The effects observed when carotids were released after occlusion for periods of such length were invariably the same, and reference to them is omitted in the rest of the protocol.

regards the eye response evoked during interference with the arterial supply of the eye ball, precisely in the same way as stimulation of the splanchnic, absolutely clinches the proof that the nerve exerts its effect by liberating epinephrin.

The amount of adrenalin necessary to reproduce approximately

the effect of a stimulation of the left splanchnic for 2 to 5 seconds was about 1 cc. of a 1:25,000 solution, i.e., 0.04 mg. An assay of the adrenalin used showed that it was 10 per cent under its nominal strength. Even assuming that the necessary quantity was only 0.02 mg., the large number of successful stimulations (at least 20), if each liberated 0.02 mg. of epinephrin, would imply a very large initial store of epinephrin in the left adrenal, (higher indeed than Elliot found in any cat), if all the liberated epinephrin came from the store. Add to this, that some of the stimulations were longer than 5 seconds, and we know that the reaction and therefore the amount of epinephrin set free increases up to a certain point with duration of the stimulation. Further, there is scarcely any doubt that the eye reactions could have been elicited a much larger number of times by stimulation of the nerves, had it been the object of the experiment to determine the maximum number of successful excitations. It is suggestive in this connection that the extremely low content of epinephrin in the right adrenal, accounted for, in accordance with Elliot's work, by the corresponding splanchnic nerve having remained intact during an anaesthesia of 6 hours duration (a good deal of ether being needed), coincided with a considerable power of the right splanchnic to liberate epinephrin in response to electrical stimulation.

The distinct shortening of the time interval between the beginning of stimulation of the left splanchnic and the beginning of the eye response seen in the observations commencing at 5.45 p.m. is probably connected with improvement in the circulation due to repeated injections of adrenalin and also of the Ringer's solution in which it was dissolved.

We take the opportunity to point out here that the denervated eye reactions not only constitute an extraordinarily sensitive test for small quantities of epinephrin circulating in the blood, but also afford the means of studying certain questions connected with the mass-movement of the blood, and especially with the collateral circulation when large vessels are blocked. The demonstration of the onward flow of blood in the inferior cava, after occlusion of the aorta, has already been referred to.



As already stated, the eye response in this animal was very easy to elicit by stimulation of the splanchnic, and very difficult to exhaust. In consequence, it was no doubt more difficult to abolish it by occlusion of the carotids than in animals in which a smaller response was obtained. The cat was exceptionally large, and in large animals the collateral circulation may be expected to be freer than in small, as witness the difference in the effect of ligating the four cerebral arteries in dogs on the one hand, and in cats and rabbits on the other.

Also the thyroids were much enlarged. The clips were put on the carotids below the level of the thyroids and a freer collateral circulation than normal may have existed distal to the clips. It may be predicted, that in different animals the effect of clamping one or both carotids, on the eye response, will vary considerably with the excitability of the epinephrin-liberating mechanism, and with the abundance of the collateral circulation. As a matter of fact, in another cat, whose response to splanchnic stimulation had never been as good as in the case just described, and had been to a certain extent exhausted by the time the observations on occlusion of the carotids were made, it was very easy to abolish the response by clamping the ipsilateral carotid alone.

In this animal, whose superior cervical ganglion had been removed 8 days before, the left carotid was clamped during stimulation of the peripheral end of the left splanchnic nerve. No eye reaction was obtained, either during the occlusion or after the removal of the clamp, although stimulation of the splanchnic was effective in control observations with the carotid free. For example, in one observation, stimulation of the splanchnic was followed by full dilatation of the left pupil in 10 seconds from the beginning of stimulation. The pupil returned to normal in 1 minute. Four minutes later the carotid was clamped, and the splanchnic stimulated for 25 seconds, without result on the denervated eye. Stimulation was then stopped and the clamp removed 15 seconds later. No eye effect was obtained after removal of the clamp.

## MASSAGE OF THE ADRENALS

It was shown by one of us (10) that when the adrenal (in the dog) was massaged, a quantity of epinephrin, easily detectable by the intestine and uterus segment reaction, was liberated into the adrenal vein. The concentration of epinephrin in blood collected from the vein, in one experiment, was estimated at 1:500,000. The reactions of the denervated eye are also obtained (in cats and dogs) when one or both adrenals are massaged after section of the corresponding or of both splanchnics. The time interval between the beginning of massage and the beginning of the eye reaction, when massage gives a good reaction, is the same, within the limits of error of our observations, as the interval when the eye reaction is elicited by stimulation of the splanchnic. In an animal in which splanchnic stimulation is causing a good eye response, even slight and momentary massage of the corresponding adrenal may be effective. The reaction is therefore due to the setting free of epinephrin just as when it follows stimulation of the splanchnics.

That the latent period of the liberation of epinephrin by massage is very short, as in the case of its liberation by stimulation of the splanchnic, is indicated by a comparison of the time interval at which the eye reactions occur with the interval after injection of adrenalin. For example, in an experiment on a dog the right adrenal was massaged and dilatation of the pupil occurred in the denervated eye in 11.2 seconds from the beginning of massage. 2.5 cc. of a 1:50,000 solution of adrenalin in Ringer's was now injected into the central end of the femoral vein, and dilatation of the pupil, which soon became maximal, was observed at 11.6 seconds.

The question may be asked whether the effect of massage is not really a mechanical stimulation of the nerve fibres in the glands. That it is something more is indicated by the fact that when after repeated stimulation, excitation of a splanchnic nerve has ceased to be followed by an eye reaction, a good reaction may still be obtained by massage of the gland. Ultimately, this fails also, and at a time when the gland still contains epinephrin, as determined by quantitative estimation after its removal.

For example, in an experiment on a cat (anesthetized with ether alone), the left splanchnic was stimulated before cutting it. The eye reaction was readily obtained (in 10; 11 and 10 seconds in three successive observation, in which the nerve was stimulated till the reaction appeared). After section of the splanchnic, stimulation of its peripheral end was followed in 11 seconds by dilatation of the pupil and retraction of the nictitating. The nerve was then stimulated in seven further observations for periods varying from 15 to 22 seconds. No effect was now produced on the eye. The electrodes were then applied to the left adrenal gland directly for 37 seconds. There was no response by the pupil or nictitating. Immediately thereafter massage of the left adrenal was performed, until a response was obtained in 12 seconds from the beginning of the massage. The eye response was maximal, and the pupil remained well dilated for more than 5 minutes, indicating that the massage had liberated a considerable amount of epinephrin. Five minutes after the first massage, the gland was again massaged for 30 seconds, without causing any further dilatation of the pupil, which had not at this time diminished to its original size. Three minutes later the splanchnic was stimulated for 18 seconds without effect. Massage of the gland was repeated in 3 minutes, till dilatation of the pupil occurred in 15 seconds from the beginning of the massage. The dilatation persisted for 10 seconds. Finally, the splanchnic was stimulated for 23 seconds without evoking any eye response.

In an experiment on a dog, the left splanchnic (in the thorax) had been stimulated 13 times, stimulation being in each case followed by good eye reactions. The average time of stimulation was 15 seconds. The time interval at which the eye-response followed the beginning of stimulation ranged from 8.2 to 11.2 seconds at different stages in the experiment. Two minutes before the effect of massage of the adrenals was tested, stimulation of the left splanchnic caused dilatation of the pupil in 8.4 seconds. A brief massage of the left adrenal then produced an eye response in 8.2 seconds. The dilatation of the pupil was good, and greater than that caused by the previous stimulation of the nerve. Five minutes later the left adrenal was massaged for 2 seconds; no effect was caused on the eye. After an interval of 2 minutes, the left adrenal was strongly massaged for 5 seconds, and dilatation of the pupil was seen 18.4 seconds after the beginning of the massage. The pupil, however, was still dilated from the first massage. One minute later the gland was massaged strongly, and full dilatation of the pupil was obtained in 22.4 seconds. The massage was still continued with



the object of exhausting the epinephrin store. The dilatation lasted  $3\frac{1}{2}$  minutes. The left splanchnic was then stimulated for 20 seconds without effect on the eye. Massage of the left adrenal now caused a very slight dilatation of the pupil after 31.8 seconds. The right splanchnic (in the thorax) was now stimulated twice for 20 seconds without effect. Massage of the right adrenal was then performed, and dilatation of the pupil occurred 11.2 seconds from the beginning of the massage.

It will be observed that massage of the right unexhausted adrenal caused a pupil reaction after a time interval of the same order of magnitude as was obtained earlier in the experiment, while the animal was in good condition, by stimulating the splanchnic; and also of very much the same length as the time interval of the reaction elicited in the first massage of the left adrenal. The conclusion can scarcely be avoided that the reason for the extraordinary increase in the length of the interval in the later massage observations on the left adrenal is due to the fact that very little epinephrin could now be liberated by massage. It therefore took a considerable time to accumulate such an effect on the pupil as comes within the limits of observation.

The left adrenal in this dog weighed 0.376 gram and contained 0.24 mg. of epinephrin. The right adrenal weighed 0.360 gram and contained 0.42 mg. of epinephrin. The difference is striking, particularly if we reflect that the right splanchnic remained intact much longer than the left, and according to Elliott, exhaustion of the epinephrin store proceeds rapidly in an anesthetized animal, in the gland whose splanchnic supply has not been divided, whereas little if any effect is produced by prolonged stimulation of the cut splanchnic nerve. Accordingly, it seems highly probable that the difference in the epinephrin content of the two glands in this experiment represents the epinephrin liberated by the vigorous massage of the left adrenal, or a portion of this difference. The great dilatation of the pupil of the denervated eye occasioned by this massage supports the idea that the amount of epinephrin so liberated was substantial.

After a gland has failed to yield an eye reaction either by



stimulation of the splanchnic or by massage, a reaction may again be obtained from it after a period of rest. But so far as our experiments on this point go, the power to respond is never so great as at first and is soon again lost.

RELATION OF THE EPINEPHRIN STORE IN THE ADRENAL TO THE  
EPINEPHRIN LIBERATED BY SPLANCHNIC STIMULATION

We have tried to determine whether there is any relation between the stock of epinephrin in an adrenal gland and the readiness with which epinephrin is given off to the blood (as tested, for example, by the minimum length of stimulation of a given strength which will yield an appreciable reaction) or the total amount of epinephrin which can be so given off in response to splanchnic stimulation or massage. The details of our observations on this point will be omitted for the present. While, as has been already mentioned, good eye reactions have been seen in animals whose adrenals at the end of the experiment were still found relatively rich in epinephrin, and poor eye reactions in animals whose adrenals at the end of the experiment contained only a small epinephrin store, we have also seen perfectly good reactions, and have been able to elicit them frequently over long periods, in animals whose adrenals when finally excised were found poor in epinephrin. It does not, at present at any rate, appear that glands with a relatively low epinephrin content necessarily give a poorer response, as tested by the eye reactions, or one more easily exhausted, than glands with a relatively high content. This statement is based merely upon the routine examination of the glands, after the experiments were finished, by the method of Folin, Cannon and Denis (1). The animals had, of course, been anesthetized for a considerable time. The epinephrin content may therefore be assumed to have been diminished to some extent. We tried in one case to bring about a high degree of exhaustion by a prolonged period of "frightening" a cat by a dog just before the experiment. But the epinephrin content was found to be fully as high as in cats in which precautions has been taken to reduce their emotional disturbance

and struggle to the minimum. The eye reaction to stimulation of the splanchnic was quite as good, and could be obtained quite as often without exhaustion, as in the run of our experiments. There is no *a priori* reason to assume that the epinephrin liberated into the blood in response to stimulation of the nerves must necessarily come entirely from the store already present in the glands. If it does come entirely from this store, it is not easy to understand Elliott's result, that stimulation of the splanchnic fails to cause any reduction in the epinephrin content of the corresponding adrenal. For nothing is more certain, than that epinephrin is given off into the adrenal vein blood during such stimulation. While it may be true that we cannot definitely assay this quantity, by comparing the eye response evoked by splanchnic stimulation with the quantity of injected adrenalin necessary to elicit the same amount of response, since we do not know for certain that the epinephrin as it passes into the circulation from the adrenals is quite the same thing as the adrenalin which we introduce from a burette, yet there is no doubt that when in the course of an experiment a splanchnic has been stimulated 15, 20 or even 50 times, each time evoking a definite eye reaction, a total amount of epinephrin must have passed from the gland large enough to be reflected in a diminution of the final content of that gland, unless in the meantime the epinephrin store was being recruited.

In a cat, which had received urethane the day before the experiment and morphine three hours before the experiment and which was then anaesthetized with urethane and ether, a very low content of epinephrin was found in the adrenals at the end of the experiment (see condensed protocol in table 8). The glands, it is true, had been massaged shortly before the animal was killed, but had yielded little epinephrin to the blood as judged by the eye reaction elicited by the massage. There is accordingly every reason to believe that the content of epinephrin was small throughout the whole or the greater part of the experiment. If the maximal dilatation of the pupil, not only that of the highly sensitive denervated eye but the other also, induced by the morphine and persisting for the  $7\frac{1}{2}$  hours for which the cat was

observed, was maintained by epinephrin from the adrenal store, it is not conceivable that at any time after observations on stimulation of the splanchnics were begun the store could have been great. Yet such reactions as were still available (retraction of the nictitating and widening of the aperture) were readily and repeatedly evoked through stimulation of the peripheral ends of the nerves. There is no doubt that many more successful stimulations could have been made. On the assumption that each of these required the liberation of an amount of epinephrin equal to the amount of adrenalin which had to be artificially introduced in order to give a similar reaction, the question again arises, as in the experiment given in table 7, whether the whole of this could possibly have come from the stored epinephrin, or whether epinephrin liberated in response to splanchnic stimulation was formed at the moment.

In another cat, a young animal weighing 1.5 kg., the left splanchnic (in the thorax) was stimulated 52 times in the course of 4 hours. No doubt a larger number of successful stimulations could have been obtained. The amount of adrenalin hydrochloride which had to be injected into the femoral vein to yield a reaction equal to the average in the splanchnic observations was at least 0.008 mg. This would be equivalent to, say 0.4 mg. for the total number of reactions. The left splanchnic had been cut  $1\frac{1}{4}$  hours after the administration of urethane, the right 37 minutes later. The right splanchnic was stimulated only 3 or 4 times, always with a positive result. The last stimulation of the right splanchnic, at the end of the experiment gave as good a reaction as those made immediately after its isolation. Both adrenals were small. The right weighed 0.104 gm. and contained 0.10 mg. of epinephrin. The left adrenal weighed 0.097 gm. and contained 0.14 of epinephrin. The small content in each case is probably due to the exhaustion of the store under the anaesthesia before the nerves were divided. The right gland, whose nerve remained longer intact, has naturally a somewhat smaller content.

But it is certainly a striking fact that the gland which had liberated enough epinephrin to cause a definite eye reaction more than fifty times<sup>2</sup> (an effect equal to that produced by 0.4 mg.

<sup>2</sup>We have since had more than 300 successful stimulations in a cat.



TABLE 8

*Condensed protocol of experiment on a cat weighing 1.5 kg. Left superior cervical ganglion excised four days previously. On the day before the experiment, the animal received 1 gram urethane and was allowed to recover.*

TIME		EYE-REACTION AFTER
		<i>seconds</i>
11.00	20 mg. morphin subcutaneously.....	
2.00	1 gram urethane, by stomach tube.....	
2.15	Etherized. Both splanchnics ligated in thorax and cut.....	
2.50	Left splanchnic stimulated.....	No effect
2.55	Both splanchnics stimulated 10 seconds.....	10.6
2.58	Both splanchnics stimulated 8.6 seconds.....	8.6
3.01	Both splanchnics stimulated (weaker current)....	9.6
3.04	Both splanchnics stimulated (weaker current)....	8.0
3.15	2 cc. adrenalin (1: 100,000) in femoral vein.....	8.6
3.25	0.5 cc. adrenalin (1: 25,000).....	8.4
3.29	2 cc. adrenalin (1: 100,000).....	9.8
3.36	Both splanchnics stimulated for 8 seconds.....	8.0
3.42	2 cc. adrenalin (1: 100,000).....	8.6
3.52	2 cc. adrenalin (1: 100,000) (with aorta clamped for 10 seconds).....	10.8
3.58	0.5 cc. adrenalin (1: 25,000) with aorta clamped for 10 seconds).....	7.4
4.12	0.5 cc. adrenalin (1: 25,000) (with aorta clamped)..	6.8
4.25	0.5 cc. adrenalin (1: 25,000) (with aorta clamped)..	9.2
4.30	Both splanchnics stimulated for 9.2 seconds.....	9.2
4.35	Both splanchnics stimulated for 10 seconds (with aorta clamped).....	10.4 (slow re- traction)
4.42	Both splanchnics stimulated for 1 second.....	11.6
4.45	Both splanchnics stimulated for 0.5 second.....	No effect
4.46	Both splanchnics stimulated for 0.5 second.....	No effect
4.47	Both splanchnics stimulated for 1 second.....	14.0 (slow re- traction)
4.48	Both splanchnics stimulated for 2 seconds.....	13.0 (slow re- traction)
4.50	Both splanchnics stimulated for 2 seconds (with aorta clamped).....	14.8 (slow re- traction)
4.52	Both splanchnics stimulated for 5 seconds.....	11.2 (good re- traction)
4.55	Both splanchnics stimulated for 5 seconds (with aorta clamped).....	11.8
5.00	Clamped aorta for 5 seconds; then stimulated splanchnics for 10 seconds (with aorta clamped)..	11.8
	Aorta released.....	15.4 (further re- traction)



TABLE 8—Continued

TIME		EYE-REACTION AFTER
		<i>seconds</i>
5.07	Clamped aorta for 20 seconds; then stimulated splanchnics for 5 seconds (with aorta clamped)...	No effect
	Aorta released after 35 seconds.....	No effect
5.09	Splanchnics stimulated for 5 seconds.....	11.2
5.13	Clamped aorta for 10 seconds; then stimulated splanchnics for 5 seconds.....	8.6
5.35	Clamped inferior cava for 35 seconds.....	No effect
5.43	Clamped aorta and cava for 30 seconds.....	No effect
5.44	Stimulated splanchnics for 5 seconds.....	No effect
5.48	Stimulated splanchnics for 10 seconds.....	15.0 (slow)
5.52	Clamped aorta and stimulated splanchnics for 10 seconds.....	15.4
5.56	Clamped cava; then stimulated splanchnics for 10 seconds.....	No effect
	Released cava after 24 seconds.....	12.4
6.00	Clamped cava; stimulated splanchnics for 10 seconds.....	No effect
	Released cava after 23 seconds.....	16.6
6.01	0.1 cc. adrenalin (1: 25,000).....	16.6
6.05	0.2 cc. adrenalin (1: 25,000) with aorta clamped....	11.6
6.10	0.1 cc. adrenalin (1: 25,000) with aorta clamped....	9.8
6.12	0.04 cc. adrenalin (1: 25,000) .....	17.0 (very slow)
6.17 to	Stimulated splanchnics for 10 seconds in 5 successive observations .....	No effect
6.27	Massaged lightly, left adrenal.....	No effect
6.30	Massaged strongly, left adrenal.....	No effect
6.31	Massaged lightly, both adrenals.....	No effect
6.32	Massaged vigorously, both adrenals.....	15.0 (slow)
6.33	Stimulated splanchnics for 10 seconds.....	No effect
6.35	0.2 cc. adrenalin (1: 25,000).....	16.8 (good re- traction)
6.40	Heart still beating well, experiment stopped. Left adrenal, weight 0.121 gm. epinephrin 0.07 mg. Right adrenal, weight 0.124 gm. epinephrin 0.07 mg.	

of adrenalin) should still have contained, if anything, more epinephrin than the gland which had only liberated enough to cause the reaction three or four times. It does not seem conceivable that the epinephrin set free could all have come from the

initial store. There is some evidence that the epinephrin set free by massage comes from the store in the adrenals, but that is quite a different thing.

#### SUMMARY

1. It is shown (on cats and dogs) that the response of the denervated eye to stimulation of the peripheral end of the splanchnic nerves, is due solely to the passage of a substance in the blood stream from the adrenals to the eyeball. For

(a) When the venous path is blocked the response fails, but appears on releasing the block, and at the same interval of time as when the vessels are free. The active substance must therefore have accumulated during the period of stimulation of the nerves behind the block.

(b) When the heart is stopped by stimulation of the peripheral end of the vagus, stimulation of the splanchnics produces no effect on the eye. But on allowing the heart to beat again, the eye response occurs at approximately the same time from the moment of reestablishment of the circulation, as the time interval between stimulation of the splanchnics and the response with the circulation going on normally. During the stoppage of the circulation, by complete cardiac inhibition, accordingly, stimulation of the splanchnics must have caused liberation of the active substance at the same point from which it starts when the splanchnics are stimulated without cardiac inhibition.

(c) When the circulation is slowed without being stopped, as by producing partial inhibition of the heart through the vagus or by hemorrhage, the interval between the beginning of stimulation of the splanchnics and the appearance of the eye response is correspondingly increased.

(d) It is possible to find a strength and duration of stimulation of the splanchnics with which no eye response will be obtained, when the ipsilateral or both carotids are clamped, but which will give a response with the vessels free. With longer or stronger stimulation, a response, but a belated one, may occur even with the carotids clamped. The abolition of the response, and its retardation, can be imitated when appropriate doses of adrenalin

are injected into the femoral vein with the carotids clamped or free.

(e) When adrenalin is injected into the left renal vein, or into the central end of the femoral vein, in suitable amount to produce an eye response approximately equal to that produced by a given stimulation of the splanchnics, the interval of time after which the response follows is sensibly the same for adrenalin injection as for splanchnic stimulation.

2. When the aorta is clamped, and the splanchnic then stimulated, a response may be obtained in the eye while the clamp is still on, or only after its removal, or both during the application and after removal of the clamp. There is some variability in this regard in different experiments. There is also a somewhat greater variability in the time interval at which the response appears, than in observations in which the splanchnics are stimulated with the vessels free, or with the veins clamped. The interpretation of these differences is discussed.

3. Circulation time measurements show that there is always more than sufficient time for a substance to have been carried in the blood from the adrenals to the eye before the appearance of the eye reactions.

4. The latent period of liberation of epinephrin from the adrenals on stimulation of the splanchnics is short since the time interval after which the eye response occurs is sensibly the same whether it is evoked by splanchnic stimulation or by the injection at the level of the adrenals of a quantity of adrenalin sufficient to elicit a response similar in character and amount.

5. The minimum period of stimulation of the splanchnics needed to liberate sufficient epinephrin to elicit a response in the denervated eye is very brief (a fraction of a second). With a current of given intensity the amount of the response increases up to a certain point with the duration of the stimulation.

6. Massage of one or both adrenals causes definite eye response in an animal in which stimulation of the splanchnics has been causing it, and at the same interval of time. When, after repeated excitations of a splanchnic nerve, the reaction on the eye ceases to be obtained, it can still in general be elicited by mas-



sage of the corresponding adrenal. But this reaction is soon exhausted.

7. Good eye reactions have been obtained by stimulation of the splanchnics in cats, in which attempts were made before the experiment to exhaust the epinephrin store of the adrenals, for example, by frightening or by administration of morphin. It did not seem that it was easier to exhaust the capacity of the splanchnic nerves for eliciting these reactions in such animals, than in animals which were guarded as much as possible against preliminary exhaustion of the epinephrin store by psychical disturbances.

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(Note added April 24, 1916.) It has since been shown by two of us (S. & R.) that sufficient epinephrin is liberated from the adrenals in cats (under ether anaesthesia) without electrical stimulation of the splanchnics to give a definite eye reaction. The experiment is done by clamping the inferior cava just above the iliac veins and also clamping the two renal veins, then stripping the cava gently upwards so as to empty it of blood above the clamp and finally clamping it above the adrenal veins. Small branches of the segment of cava have been previously tied. The pocket is allowed to fill with blood from the adrenals. When the clamps are removed the eye reactions are obtained at the same time interval as when the splanchnics are stimulated with the vessels free. After division of the splanchnics (in the thorax) the blood collected in the pocket does not affect the eye unless the splanchnics are stimulated.





## THE RÔLE OF THE LIVER IN ACUTE POLYCYTHAEMIA

### III. THE RELATION OF PLASMA VOLUME TO THE NUMBER OF ERYTHROCYTES PER UNIT VOLUME OF BLOOD

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There are at the present time, certain generally accepted ideas concerning the number of erythrocytes per unit volume of blood, which will bear a more careful consideration. One view is that an increased number of erythrocytes per unit volume of blood, is an index of a concentrated blood. Furthermore if fluid is lost from the blood, a concentration must take place, with a relative increase in number of erythrocytes. We find these ideas expressed in the clinic, where sweating, marked watery diarrhoea, etc., are spoken of as concentrating the blood, and in more exact work in the laboratories where the total solids of the blood are used as an index of its "concentration," meaning in many instances that the plasma volume varies with the total solids of the blood. The investigations reported in this communication have shown however that these views must be somewhat modified.

It has been shown by one of the present authors (P. D. L.) (1), that the intravenous injection of epinephrin in the normal animal causes an increase in the number of erythrocytes per unit volume of blood, and that the liver is the organ which is responsible for these changes. A decrease in plasma volume of sufficient magnitude to account for a large portion of this increase in number of red blood corpuscles by a concentration of the blood was experi-

mentally demonstrated. And finally it was shown that if the hepatic artery is ligated previous to the injection of epinephrin, no increase in number of erythrocytes per unit volume of blood takes place.

Knowing that epinephrin causes an increase in number of erythrocytes per unit volume of blood in the normal animal, and no change in number if the hepatic artery be first ligated, it was considered of interest to determine what change if any in the plasma volume occurs in the latter condition.

If the generally accepted view that a loss of plasma from the blood causes a concentration and a relative increase in number of erythrocytes per unit volume of blood be correct, one would expect in the condition in which the hepatic artery was ligated and no increase in number of erythrocytes per unit volume of blood took place after the injection of epinephrin, that no change in plasma volume would occur. The following experiments show the invalidity of this reasoning.

The method of determining plasma volume is fully described in an article by Keith, Rowntree, and Geraghty (2), to which the reader is referred. The principle of this method is to determine the dilution of a known amount of dye in the plasma of the blood, by colorimetric methods, from which the plasma volume may be calculated, the total blood volume being estimated from the plasma volume and the haematocrit readings. The red counts were done as previously described. The epinephrin solution injected, was freshly prepared from the same pure powdered epinephrin used in the previous experiments here mentioned.

*Experiment 205.* January 28, 1916.

Dog. 11.65 kilo.

10.05. Ether.

Operation performed exposing the liver and cutting and tying all vessels to the liver except the hepatic artery (the branches of which were also ligated), and the portal vein.

11.24. Hepatic artery clamped.

11.27. Red count, 7,576,000. Haematocrit. Erythrocytes, 41 per cent. Plasma volume, 656 cc. Total blood volume, 1112 cc.

11.48. Epinephrin 0.9 mg. per kilo intravenously.

- 12.00. Red count, 7,624,000. Haematocrit. Erythrocytes, 46 per cent. Plasma volume, 535 cc. Total blood volume, 990 cc.  
 12.07. Clamp removed from hepatic artery.  
 12.17. Red count, 8,280,000.  
 12.24. Red count, 8,336,000.  
 12.32. Red count, 8,040,000. Haematocrit. Erythrocytes, 42 per cent. Plasma volume, 512 cc. Total blood volume, 882 cc.

*Experiment 165.* November 15, 1915.

Dog, 9.7 kilo.

- 10.05. Ether. Operation similar to that performed in experiment 205.  
 11.45. Hepatic artery clamped.  
 11.59. Red count, 7,360,000. Haematocrit. Erythrocytes, 40.2 per cent. Plasma volume 431 cc. Total blood volume, 720 cc.  
 12.24. Epinephrin 0.9 mg. per kilo intravenously.  
 12.30. Red count, 7,144,000. Haematocrit. Erythrocytes 42.7 per cent. Plasma volume, 247 cc. Total blood volume, 430 cc.  
 12.36. Clamp removed from hepatic artery.  
 1.05. Red count, 8,456,000.

*Experiment 167.* November 19, 1915.

Dog. 18.60 kilo.

- 11.35. Ether. Same operation as in the above experiment.  
 1.15. Hepatic artery clamped.  
 1.23. Red count, 9,456,000. Haematocrit. Erythrocytes 50.5 per cent. Plasma volume, 886 cc. Total blood volume, 1790 cc.  
 1.28. Epinephrin 0.9 mg. per kilo intravenously.  
 1.58. Red count, 8,960,000. Haematocrit. Erythrocytes 51.0 per cent. Plasma volume, 791 cc. Total blood volume, 1614 cc.  
 2.26. Red count, 9,584,000. Haematocrit. Erythrocytes 53.1 per cent. Plasma volume, 800 cc. Total blood volume, 1706 cc.

*Table of the percentage changes in the red count, plasma volume, and total blood volume in normal animals, following the injection of epinephrin<sup>1</sup>*

ERYTHROCYTES	PLASMA VOLUME	TOTAL BLOOD VOLUME
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
28+	13.3—	3.97—
23+	19.3—	14.00—

<sup>1</sup> See note (1), p. 198.



*Table of the percentage changes in the red count, plasma volume, and total blood volume in animals in which the hepatic artery was ligated previous to the injection of epinephrin.*

ERYTHROCYTES	PLASMA VOLUME	TOTAL BLOOD VOLUME
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
0.6+	18.4—	11.0—
1.6—	40.0—	42.0—
5.0—	10.7—	9.1—

Comparing the change of plasma volume in these experiments with those in the normal animal after the injection of the corresponding amount of epinephrin per body weight, we see that they are roughly the same.

In the experiments in which the hepatic artery is tied previous to the injection of epinephrin we have as a result of the injection, an increase in the red count of 48,000, in one instance, while in the other two experiments there is a decrease of 216,000 and 496,000 respectively. These changes may be considered negligible on account of the errors of the method, except in the last instance in which there is a slight decrease in number of erythrocytes per unit volume of blood.

Compared with these insignificant changes in number of red blood corpuscles per unit volume of blood the plasma volume is seen to decrease 18.4, 40, and 10.7 per cent.

A condition has thus been experimentally demonstrated in which fluid is lost from the blood, and in which there is no increase in number of erythrocytes per unit volume of blood. This is theoretically impossible without there being a loss of red corpuscles from the circulation, either by a destruction of these, or a storing away of them somewhere out of the generally circulating blood.

The discussion of the fate of these corpuscles, will be taken up in a paper about to be published by one of us (P. D. L.), but it may be of interest to point out that there is considerable evidence to favor the view that these red corpuscles are stored in the liver. The fact previously observed, and again seen in these experiments, that opening the hepatic artery sometime after the injection of epinephrin causes an increase in number of red blood

corpuscles to take place, together with lack of evidence of red cell destruction, and the fact that polycythaemia may be repeatedly produced in the same animal, suggests strongly that the red cells are not destroyed but temporarily stored in the capillaries of the liver.

#### SUMMARY

1. It has been demonstrated that when the hepatic artery is ligated, the intravenous injection of epinephrin causes a decrease in plasma volume, without an increase in the number of erythrocytes per unit volume of blood.

2. It has been experimentally shown that plasma volume and the number of erythrocytes per unit volume of blood may vary independently of one another.

- (1) LAMSON, P. D.: *Journ. of Pharm. and Exp. Therap.*, vol. vii, July, 1915, pp. 169-224.
- (2) KEITH, ROWNTREE, AND GERAGHTY: *Archiv Int. Med.*, vol. xvi, 1915, pp. 547-576.



## THE ACTION OF CERTAIN VOLATILE OILS ON ISOLATED INTESTINAL SEGMENTS

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In 1913 Macht (1) published the results of a series of experiments on the action of the emmenagogue oils on isolated uterine strips and we have undertaken, by a similar method, to determine the action of these, and some other oils, on isolated intestinal segments. The extensive use of certain oils of the volatile series as carminatives made it appear probable that they exert some influence on the motor mechanism of the intestinal tube, and our findings, though essentially preliminary and incomplete, indicate the general character of the action of these oils. Salant and Mitchell (2) in 1915 published the results of their investigations of the influence of oil of chenopodium on intestinal contractility, having studied the action of this oil on intact animals as well as on isolated intestinal segments. We have included the oil of chenopodium in our studies and our results agree with those of Salant and Mitchell as to its action on isolated intestinal segments, but we have not yet made any studies on intact animals.

### METHODS

Our studies were made on segments of small intestine only, from dogs, cats and rabbits. The dogs and cats were killed by administering concentrated chloroform vapor, the smaller animals by the blow of a hammer on the back of the head. The abdomen was opened at once and a segment of bowel was grasped by two pairs of forceps about 6 cm. apart. Two fine needles carrying ligatures were passed next through the wall of this segment between the forceps and about 2.5 to 3 cm. apart. These ligatures were loosely tied and then the portion of bowel between and a



little beyond them was removed with a pair of sharp scissors and placed in warm Ringer's solution and the contents carefully washed out by means of a pipette. One of the ligatures was then fastened to the lower end of a bent glass tube which carried the oxygen supply to the bottom of the vessel in which the segment was suspended, and the other was attached above to a spring lever which extended over the vessel to a slowly revolving drum. Extreme care was taken not to manipulate the segment of bowel removed, in fact, it was not touched except by the needle and thread which pierced it. The vessel in which it was suspended had a capacity of a little over 250 cc. and was kept at a uniform temperature by being placed in a large water bath heated by an ordinary Bunsen burner. Various solutions were used in the vessel containing the segment, Ringer's, Locke's and Tryodes': but very little difference was observed in the activity of segments in the different solutions. The temperature giving best results varied somewhat with the kind of animal used. Segments from dogs gave best results at a temperature of 38 C., those from cats required a slightly higher temperature, while with the segments from rabbits a temperature of 40 C. was found most satisfactory. The tension of the ligatures suspending the segment had to be carefully adjusted in each case. If too great, a gradual relaxation with lessened amplitude of movement resulted, while if not enough, a gradual shortening of the segment and consequent downward movement of the record resulted. Contraction of the segment was recorded by a downward movement of the writing lever, relaxation by an upward movement.

With the technic employed the segment almost always began active movements at once, which would continue for several hours. Usually half an hour was allowed, to obtain the proper adjustment of tension and secure a fairly uniform series of contractions before any drug was applied. The type of contractions varied considerably under very slight changes of temperature or tension, frequently even without any observable change in conditions. These apparently normal variations had to be carefully excluded as a possible source of error in our results of the action of drugs.

A definite type of contraction was observed for each species of animal used. The movements of segments from dog's intestine showed marked irregularity, both as to the pendular movements and the slower tonus waves of contraction and relaxation. Quite frequently the pendular movements would cease entirely for a time and the long tonus waves become more marked (see fig. 19). Cat's intestine showed more regularity of the pendular movements with but moderate change in tonus (see fig. 11). The rabbit's intestine showed marked regularity of the pendular movements with little or no change in tonus. The point of maximum relaxation in the record of the rabbit's intestinal movement was usually represented by a straight line on the tracing, while the point of maximum contraction showed rhythmic variations (see fig. 29). The drugs used were made up into emulsions of a uniform strength and were kept warm so as not to change the temperature of the Ringer's solution when added to it. The quantity required to make a definite percentage of the drug in the solution was added by means of a finely graduated pipette, and the stream of oxygen bubbling through soon mixed it quite thoroughly.

The following oils were studied in this manner, *oleum hedeomae* (pennyroyal), *oleum tanacetii* (tansy), *oleum absinthii* (wormwood), *oleum terebinthinae* (turpentine), *oleum rutae* (rue), *oleum anisi* (anise), *oleum sabinae* (savine), and the *stearopten thymol*.

#### RESULTS

The results may be seen from the accompanying figures which are numbered according to their order in the series of experiments made. In dilutions of 1 to 5000 each of these oils, including thymol, caused lessened movement of the intestinal segment. The result varied as to the drug used and also as to the animal, but in all cases with this drug strength, lessened pendulum movements and relaxation of the segment occurred. In dilutions of 1 to 10,000, similar results were observed but less marked and in some instances very slight. In greater dilutions the results varied markedly, some oils causing more or less marked relaxa-

tion, others just as pronounced and distinct shortening, though seldom increase of pendulum movements. The increased contraction (or tonus) was most marked in the case of oil of anise which invariably caused very decided and prolonged shortening of the segment in dilutions of 1 to 50,000 or even greater, (see figs. 31 and 35). Oil of turpentine in the same dilutions produced similar results, but in greater concentrations, such as 1 to 17,000 or more, produced relaxation, (see fig. 12). Oil of wormwood acted in a similar manner to turpentine but somewhat less efficiently. It did not produce contraction in segments from rabbits but did in the case of dogs and cats, (see fig. 11). The oil of rue produced slight increase in pendulum movements in the rabbit in solutions of 1 to 50,000 but stopped these movements when the concentration was increased, (see fig. 29). In segments from dogs and cats, oil of rue produced only relaxation in the concentrations used. The oil of pennyroyal in similar dilutions sometimes produced slight increase of tonus and movement, but not well marked, and more frequently produced only relaxation. The oil of tansy invariably caused relaxation in all dilutions tried. Its relaxing influence was the most marked of the series (see figs. 8 and 19). The oil of savine and the stearopten thymol produced only relaxation and lessened movement. The oil of tansy in concentrations of 1 to 10,000 antagonized the action of such drugs as physostigmine and pilocarpine, causing complete relaxation, (see fig. 8). In a similar manner the oil of rue in 1 to 17,000 solution antagonized the constricting effect of oil of anise in 1 to 25,000 solution, (see fig. 31.) The effect produced by any of these oils could easily be removed by placing the segment of intestine in fresh Ringer's or Locke's solution uncontaminated by any drug (see fig. 19). This shows that no permanent damage to the motor mechanism is caused by them. After relaxation produced by most of these oils, a prompt response to physostigmine or pilocarpine can usually be obtained by using a somewhat greater concentration of the drug than is necessary in the case of untreated segments. After oil of tansy, however, this result is not so easily obtained and sometimes cannot be produced.



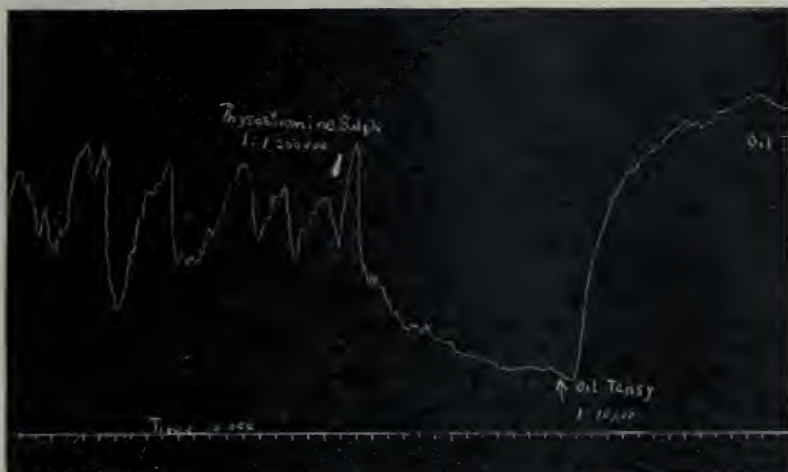


FIG. 8. DOG'S INTESTINE IN RINGER-LOCKE SOLUTION  
This shows the action of physostigmine, overcome by oil of tansy.

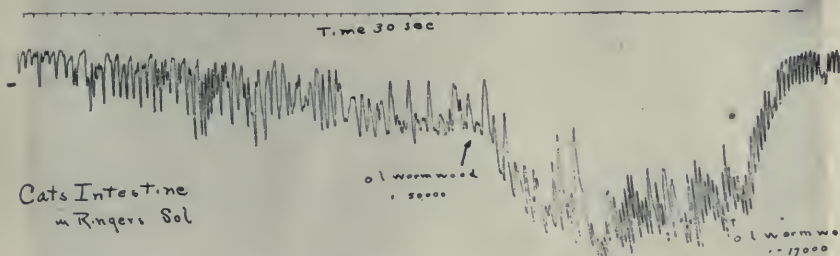


FIG. 11. CAT'S INTESTINE IN RINGER'S SOLUTION  
This shows the action of oil of wormwood in weak solutions, (1 to 50,000) and the opposite effect of stronger solutions.





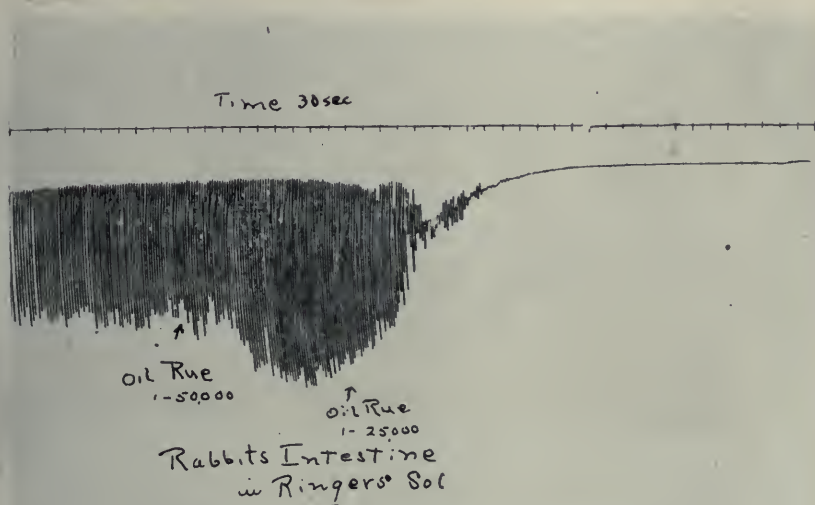


FIG. 29. RABBIT'S INTESTINE IN RINGER'S SOLUTION

This shows slight stimulation from oil of rue, 1 to 50,000 and complete cessation of movement from the same oil, 1 to 25,000.



FIG. 31. DOG'S INTESTINE, USED IN A PREVIOUS EXPERIMENT, AFTER BEING PLACED IN FRESH RINGER'S SOLUTION

This shows the action of oil of anise and the antagonism of oil of rue.

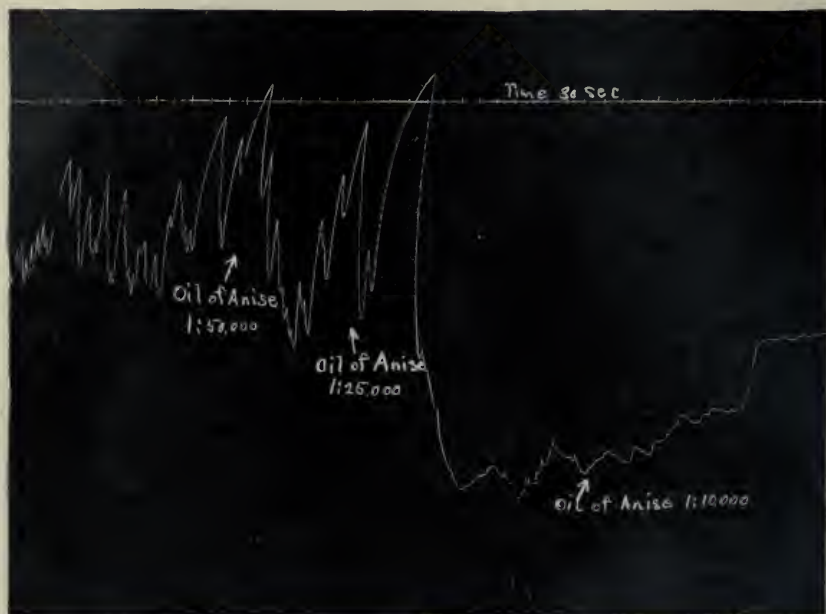


FIG. 35. DOG'S INTESTINE IN RINGER'S SOLUTION

This shows the effect of oil of anise in solutions of 1 to 50,000 and 1 to 25,000. A stronger solution (1 to 10,000) did not produce very marked relaxation.

#### CONCLUSIONS

As a result of the experimental work so far performed by us we are led to conclude as follows:

1. Of the volatile oils studied, a number produce quite marked stimulation of isolated intestinal segments when applied in dilutions of 1 to 50,000 to 1 to 25,000. This is especially true of oil of anise, oil of turpentine and oil of wormwood.

2. All of the oils studied produce relaxation of intestinal segments when applied in dilutions of 1 to 5000 to 1 to 10,000.

- (1) The Journal of Pharmacology and Experimental Therapeutics, vol. iv, no. 6, 1913.
- (2) The American Journal of Physiology, vol. xxxix, no. 1, 1915.

## ON THE PHARMACOLOGY OF THE URETER

### II. ACTION OF DRUGS AFFECTING THE SACRAL AUTONOMICS

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In a preceding paper (1) the author has described a simple and convenient yet adequate method of studying the action of drugs on the ureter, and has discussed in full the effect of epinephrin and ergotoxin on that organ. These drugs are universally regarded as typical representatives of pharmacological agents which have an affinity for or exert their action on the myoneural junctions or nerve endings of the thoracico-lumbar autonomies or so-called "true sympathetics." Quite distinct in their physiological and pharmacological behavior, from the "true sympathetics," as is well known, are the nerve fibers and nerve endings of the cranial-bulbar-sacral autonomies or the so-called "parasympathetic" nervous system (Langley). A large number of drugs have been found to be specific poisons for the parasympathetic myoneural junctions or nerve endings, some stimulating, others paralyzing them. Many organs are furthermore known to be innervated by two sets of fibers one belonging to the true sympathetic and the other to the parasympathetic system. It was, therefore, both natural and logical, after a study of epinephrin and ergotoxin to inquire into the behavior of the ureter towards the other group of drugs.

The principal members of the group of drugs affecting the sacral autonomic nerve endings are pilocarpin, physostygmmin, muscarin and cholin on the one hand, and atropin on the other; the first ones generally stimulating them, the last one paralyzing them.



## METHOD OF STUDY

The method employed was chiefly by means of rings of pig's and other ureters, and longitudinal strips of slit ureters, as already described. The same results were obtained with rings of human ureters whenever obtainable. In addition to these, some observations were also made on intact ureters in situ in living animals, as will be described later.

## ACTION OF PILOCARPIN

Pilocarpin in small quantities was found to exert a stimulating or pressor effect on the ureter. Doses of 1 or 2 mgs. of pilocarpin hydrochloride in 25 cc. of Locke's solution, increased the

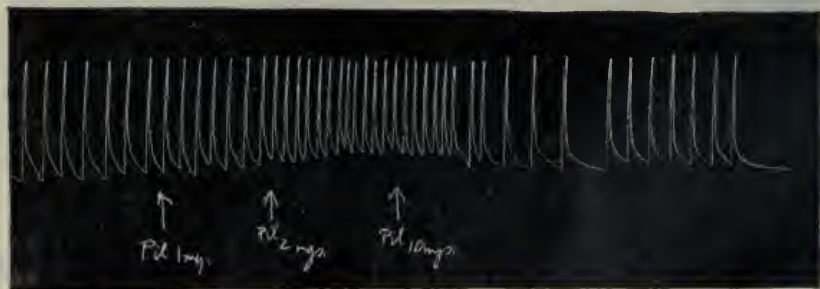


FIG. 1. ACTION OF PILOCARPIN

Pig's ureter six hours after death of animal.

rate and strength of its contractions and also heightened its tonus. Large doses of the drug, e.g., 10 mgs. in 25 cc. of solution exhibited a paralyzing effect (fig. 1).

## ACTION OF PHYSOSTYGMIN AND MUSCARIN

For the study of physostygmmin the hydrochloride was employed. For the muscarin effect both the pure alkaloid, and an aqueous extract of the toad-stool amanita muscaria were employed. Both physostygmmin and muscarin proved to be powerful stimulants of the ureter, increasing its tonicity and the rate or force of its contractions, as may be seen from the tracings. Un-

like pilocarpin, however, these drugs even in large doses never paralyzed the organ (figs. 2 and 3. Also figs. 8 and 9).

#### EFFECT ON LONGITUDINAL STRIPS

The action of pilocarpin, physostygmmin, and muscarin were also studied on longitudinal strips. The effect here was also to increase the tonicity of the smooth muscle. As the longitudinal muscles of the ureter, however, have been found to exhibit no

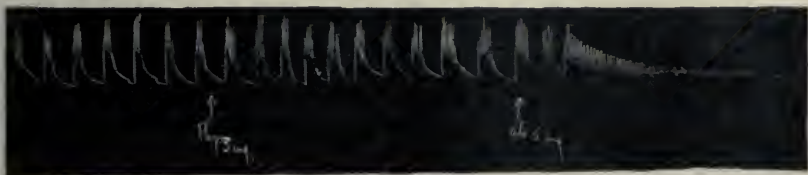


FIG. 2. ACTION OF PHYSOSTYGMIN AND ATROPIN  
Pig's ureter eighteen hours after excision.



FIG. 3. PIG'S URETER

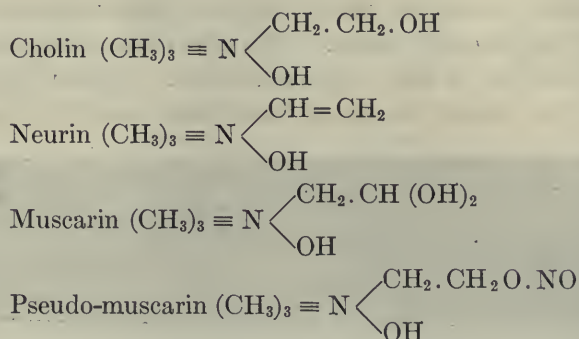
One ring, twelve hours old. Effect of 0.5 cc. of extract of *Amanita muscaria*. One gram of dried fungus was triturated with 10 cc. of cold normal saline and filtered.

rhythmical contractions, or at best but very slow and occasional ones, the effect of the drugs was not so strikingly demonstrable as it was on the ureteral rings.

#### ACTION OF CHOLIN AND "SYNTHETIC MUSCARIN"

These two bodies are chemically closely related to muscarin, as will be seen from the subjoined formulae. The so-called synthetic muscarin or pseudo-muscarin was first made by Schmiede-

berg and Harnack, (2) who thought it to be identical with natural muscarin, but it was soon found that it differed from the natural alkaloid in some respects physiologically, and recently Ewins (3) has shown that pseudo-muscarin is a nitrous ester of cholin.



The cholin used in the present investigation was a very pure specimen of synthetic cholin hydrochloride obtained from Prof. Reid Hunt of Harvard University. The "synthetic" muscarin was a preparation made by Grüber.

Both cholin and pseudo-muscarin were found to behave qualitatively like muscarin, but *quantitatively* to be much weaker. Small quantities (1 to 10 mgs.) produced no detectable change in the contractions of the ureteral ring. Larger quantities (10 to 30 mgs.), however, stimulated it. Ten to twenty milligrams of cholin or of synthetic muscarin were required to produce the effect given by 1 mg. of true muscarin. The results are well illustrated by the tracings (figs. 4 and 5).

#### ACTION OF ATROPIN

In regard to this alkaloid there is a wide divergence of opinion among the few earlier observers who studied its effect on the ureter. Thus, Lucas (4) found only negative results. Protopopoff (5), however, studying the action of atropin by direct observation of the ureters in laparotomized animals, states that the effect of atropin is two-fold, depending on the dose—small doses, increasing the contractions, larger doses inhibiting them. In

the present investigation, after numerous experiments it was found that small doses (1 to 5 mgs.) of atropin *do*, sometimes, but not always, produce a primary stimulation of the ureteral contractions. This effect is, however, not constant and generally after a longer or shorter interval of time passes into an inhibitory stage. Larger doses of atropin (solution of 0.01 per cent or more), produced an inhibition or paralysis of the ureteral movements in every case (figs. 6 and 7). It was further found, as



FIG. 4. RING OF PIG'S URETER

Twelve hours after death of animal. Effect of different doses of cholin. Contraction = up-stroke.



FIG. 5. RING OF PIG'S URETER

Twenty-four hours after death of animal. Action of pseudomuscarin or synthetic muscarin.

might have been expected, that atropin antagonized the action of pilocarpin, physostygmmin, muscarin, cholin, and pseudomuscarin (figs. 2 and 8). The inhibitory effect of atropin also antagonized the tonic action of epinephrin (fig. 9). The action of atropin is illustrated by the following tracings.

#### ANALYSIS

From the above experiments it is seen that pilocarpin, physostygmmin, and muscarin, stimulate the ureter, and that atropin in



sufficient doses antagonizes the action of these drugs and inhibits the ureteral contractions.

Inasmuch as the muscarin group of drugs, on the one hand, and atropin on the other, are generally recognized as exercising a selective action on the myoneural elements of the "parasympa-



FIG. 6. RING OF PIG'S URETER

Twenty-four hours after excision. Primary stimulation of frequency of contractions and subsequent inhibition after atropin.



FIG. 7. RING OF PIG'S URETER

Eighteen hours after excision. Slow drum. Contractions 6 per minute. Showing inhibitory effect of 1 mg. of atropine sulphate in 30 cc. Locke.

thetic nervous system, in contradistinction of the true sympathetic, the present findings seem to give pharmacological evidence of the existence of sacral autonomic nerve elements in the ureter, and this, agrees with the findings of anatomists. In order to establish more definitely the mode of action of the above drugs,

as compared with that of epinephrin, their effect was studied after ergotoxin. Ergotoxin, it has been pointed out in the previous communication, paralyzes the motor elements of the true sympathetics, and in that way inhibits the action of epinephrin.

Experiments were made to determine whether such an inhibition occurs after ergotoxin in case of physostygmmin. It was found



FIG. 8. RING OF PIG'S URETER

Twenty-four hours after excision. Showing inhibition by atropin and antagonizing action of muscarin (1 cc. of aqueous extract of fungus).

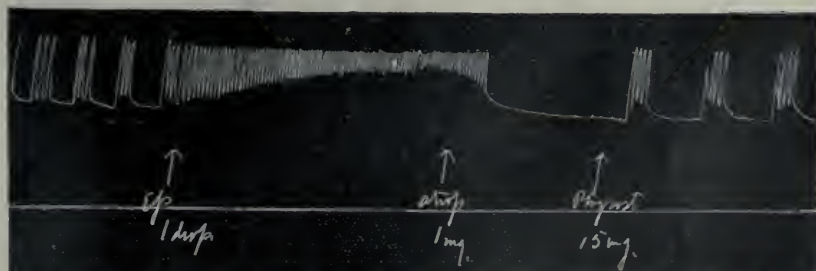


FIG. 9. RING OF PIG'S URETER

Twenty-four hours old. Showing stimulation by epinephrin 0.1 mg. in 50 cc. Locke. Inhibition by atropin, 1 mg. and resuscitation by physostygmmin 15 mgs.

that, ergotoxin does *not* inhibit or interfere with the action either of pilocarpin, physostygmmin, or muscarin. This is to be regarded as a further evidence that the drugs exert their pressor effects on the ureter, through some different point of attack from that affected by epinephrin (fig. 10).

## OBSERVATIONS ON INTACT ANIMALS

In order to determine whether the behavior of the ureter toward drugs as observed *in vitro*, is generally speaking also the same in the living body, observations were made of the actual movements *in situ*, in living animals under anesthesia. For this purpose rabbits were found to be the most suitable animals, as their ureters are easily exposed, and, not being ensheathed in an excess of fat, their movements can be easily studied. The animals

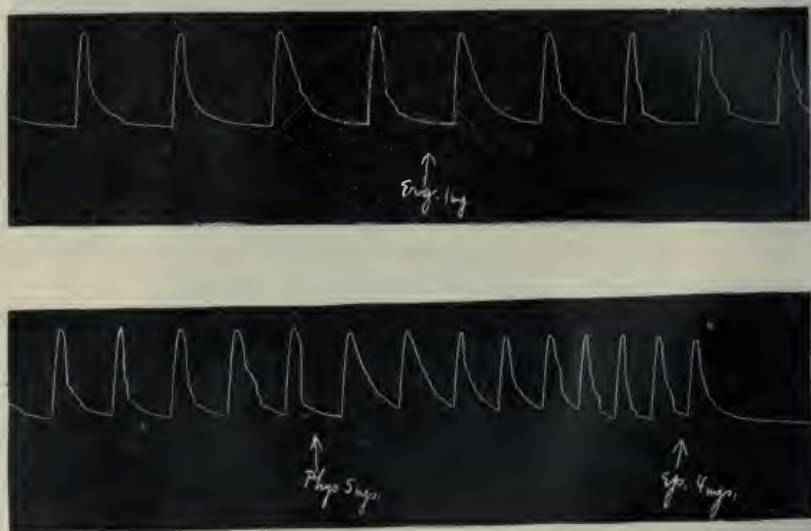


FIG. 10. RING OF PIG'S URETER

Twelve hours old. Showing how ergotoxin does not inhibit the action of physostygmmin, but does inhibit action of epinephrin.

were anesthetized with paraldehyde; kept warm with a blanket and an electric pad; the abdomen incised; the intestines gently pulled aside with gauze soaked in warm saline solution, and the ureteral movements observed at intervals for a few minutes at a time, in order not to chill the viscera. In this way the movements of the ureters, both normal and after the administration of drugs, could be studied very conveniently. The left ureter is generally better exposed, but the right one can also be seen by

pulling aside the descending colon and rectum. It was found that pilocarpin and physostygmmin stimulated the ureteral movements, whereas atropin in small doses, had little effect, but in large doses inhibited the ureters. In this respect the ureters are much less sensitive than the intestines to the effect of atropin. Muscarin was not tested on account of its cardiac effect. The following protocols illustrate some of the findings.

*Experiment. March 9, 1916*

Rabbit weighing 1250 grams; anesthetized with paraldehyde, and kept warm; the abdomen having been opened the ureters were observed in situ and their contractions noted.

Normal contractions: three to four per minute.

Injected subcutaneously 1 mg. of pilocarpin hydrochloride. Ureteral contractions are increased to five to six per minute.

Applied a few drops of pilocarpin solution (0.01 per cent) directly to one of the ureters; contractions increase to eight per minute.

Injected subcutaneously 1 mg. of atropin sulphate; ureteral contractions stop.

*Experiment. March 23, 1916*

Rabbit, weighing 1750 grams. Paraldehyde anesthesia, the same as above.

Right ureter; normal contractions, three to four per minute.

Left ureter; normal contractions, three to four per minute.

Applied a few drops of solution of physostygmmin hydrochloride (0.1 per cent) to right ureter. The drug is quickly absorbed by the peritoneum as can be seen by general increase in intestinal peristalsis.

Ureteral peristalsis on both sides, after physostygmmin increases to six to seven per minute.

Injected atropin sulphate subcutaneously 1 mg. Ureteral contractions, two per minute.

Applied atropin (a few drops of 0.1 per cent solution) to peritoneum; ureteral contractions stop.

PRACTICAL CONSIDERATIONS

The behavior of the alkaloids just described is not only of pharmacological importance, but is also not devoid of practical interest. Thus the inhibitory effect of atropin might suggest its



administration in cases of ureteral colic, but the present investigation shows that such an effect would be produced only by comparatively large amounts of the drug, far in excess of the therapeutic dosage. Other drugs, in particular papaverin, as will be shown in a later paper, will reduce the tonus and inhibit the contractions of the ureter much more efficiently and safely (6).

Again the action of pilocarpin and physostygmmin studied above explains some of the untoward symptoms met with in patients after the exhibition of these drugs. In the days when jaborandi was freely employed in practice unpleasant by-effects on the urinary apparatus were often noted. Lewin (7) states that tenesmus, strangury, and colicky pains, and other such complications occurred in 40 per cent of patients. The explanation of such phenomena and particularly of the colicky pains can be clearly grasped, from a perusal of the following protocol in which an excessive dose of pilocarpin was injected in a rabbit.

*Experiment. March 31, 1916*

Young Rabbit, weighing 900 grams

- 1.30 p.m. Introduced through stomach tube 2 cc. of paraldehyde well diluted with water.
- 2.00 p.m. Animal anesthetized, in good condition, kept on warm table, and covered with blanket.
- 2.10 p.m. Abdomen incised and ureters observed in situ.  
Right ureter; three to four contractions per minute.  
Left ureter; four to five contractions per minute.
- 2.15 p.m. Injected rapidly in ear vein 1.2 mgs. of pilocarpin hydrochloride. This was a toxic dose. The animal was almost immediately salivated profusely; there was a violent intestinal peristalsis and purging; and a *marked spasm of the bladder and ureters* was noted. Heart was slowed.
- 2.30 p.m. Slight recovery, though still depressed.  
Right ureter; six contractions per minute.  
Left ureter; six contractions per minute.
- 2.35 p.m. Injected an excess of atropin sulphate (5 mgs.) in vein.  
Intestinal peristalsis stops immediately.  
Ureteral peristalsis stops.
- 2.45 p.m. Animal dies from heart failure.

## SUMMARY

1. Pilocarpin, physostygmín, muscarin, pseudo-muscarin, and cholin, in suitable doses all stimulate the rate and force of the ureteral contractions and increase the tónus of the excised ureter. Large doses of pilocarpin may, however, secondarily paralyze that organ.

2. The pressor action of pilocarpin, physostygmín and muscarin, is not inhibited by previous exhibition of ergotoxin.

3. Atropin in sufficient (rather large) doses, inhibits the contractions and decreases the tonicity of the ureter. Small doses of atropin may produce, though not invariably, a primary stimulation of the ureteral contractions.

4. The same effects as in the ureteral preparations described by the author, were noted in rabbits by observing the ureters *in situ*, after administration of pilocarpin, physostygmín, and atropin.

5. The behavior of the ureter towards the drugs studied gives pharmacological proof of its innervation by the sacral autonomic, and is also of some practical interest.

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## THE INFLUENCE OF SALICYLATE ON METABOLISM IN MAN

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It has long been believed that the administration of sodium salicylate causes an increase in the nitrogenous metabolism and an increased output of sulphur, phosphorus and uric acid has also been shown by several investigators to follow the administration of this drug.<sup>2</sup> Practically no observations have been made, however, on the effect of salicylates on the respiratory metabolism; the only experimental work on this subject being that of Singer<sup>3</sup> who reports an increased oxygen consumption in rabbits to whom toxic doses of acetyl salicylic acid had been given.

In the present paper we wish to present the results obtained in a study of three men on whom we have made observations concerning the effects produced by the ingestion of sodium salicylate on the urine, faeces, blood, and on the respiratory exchange.

The subjects of our experiments were adult male patients in the surgical service of the Massachusetts General Hospital. During the experimental period they were in charge of a special nurse, trained in metabolism work, who had entire charge of the prepa-

<sup>1</sup> Henry P. Walcott Fellow in Clinical Medicine, Harvard University.

<sup>2</sup> See particularly: Komagawa: *Virchow's Arch. f. path. Anat.*, cxiii, p. 202 (1888); Goodbody: *Journ. Physiol.*, xxv, p. 399 (1900).

Wiley (*Bull. 84, part II, Bureau of Chemistry, U. S. Dept. of Agriculture*) states that the administration of sodium salicylate tends to diminish the excretion of uric acid and total nitrogen in the urine, a finding the opposite of that reported by all other investigators of this problem. While the conclusions of Wiley are based on observations made on a large number of subjects, the general organization of the experiments was such as to make these conclusions peculiarly unconvincing.

<sup>3</sup> *Arch. f. gs. Physiol.*, lxxiv, 527 (1901).



ration, weighing and serving of all food, and of the collection of the excreta. The diets used were selected both qualitatively and quantitatively to meet the tastes of the individual subjects, who were required to eat all the food served them. The faeces belonging to the different periods were marked off by means of charcoal.

Subjects II and III remained in bed throughout the entire period of the experiment, subject I spent six or eight hours each day in a chair.

For the analytical work the following methods were used: Nitrogen in urine and faeces, Kjeldahl method. Ammonia, Folin-Macallum method.<sup>4</sup> Creatinine and creatin, Folin's micro methods.<sup>5</sup> Uric acid, Folin-Denis method.<sup>6</sup> Phosphates, by the uranium acetate titration.

The respiratory exchange was determined by means of Benedict's<sup>7</sup> Universal Respiration apparatus. The subjects were studied in the "nuchtern" condition, lying flat, at complete muscular rest. The experiments were started about 8 a.m. after a half-hour's resting period, and three ten minute periods were then obtained. The spirometer type of apparatus was used, and the subjects breathed through a mouthpiece.

The basal metabolism was calculated by indirect calorimetry from the oxygen absorption, and the calorific value of oxygen for the respiratory quotient obtained. In the tables the basal metabolism is expressed in calories per square meter of body surface, per hour. The surface area was determined by Du Bois' height, weight, or linear formula.<sup>8</sup>

Subject I, Mr. F, a man 28 years old and weighing 61 kilos, was convalescing from a fracture of the femur which he had sustained about seven weeks before the beginning of the experiment.

Our experiment was divided into three periods: a fore period

<sup>4</sup> Journ. Biol. Chem., 11, p. 523 (1912).

<sup>5</sup> Ibid., 17, p. 467 (1914).

<sup>6</sup> Ibid., 14, p. 95 (1913).

<sup>7</sup> Benedict, F. G. Ein Universalrespirationsapparat. Deutsch. Arch. f. klin. Med., cvii, 160, 1912.

<sup>8</sup> Du Bois, E. P. Personal Communication. See also Proceedings of the Soc. for Exp. Biology and Medicine, xiii, p. 77, (1916).

of six days (Period I), a period of four days during which sodium salicylate was administered (Period II), and an after period of four days (Period III). The urine and faeces passed during the first three days of the first period were discarded; only the excreta passed on the last three days of this period being analysed.

During the entire experimental period the daily amount and quality of the food remained unchanged. The daily ration consisted of 300 grams of bread, 75 grams of butter, 6 eggs (selected to weigh 60 grams gross), 150 grams of banana, 25 grams of sugar, 950 cc. of whole milk, 250 cc. of 15 per cent cream, and 50 grams of wheat meal.

At the beginning of the experiment quantities of bread, butter, and wheat meal, sufficient to last throughout the work were secured and placed in air tight containers in cold storage. The milk, cream, eggs and bananas were taken daily from the usual hospital supply. The milk and cream were analysed at frequent intervals for protein and fat and found to be extremely uniform in composition.

The daily portion of sodium salicylate was administered in three equal doses and unless specified otherwise was given immediately after meals.

TABLE I  
*Subject I, Mr. F.*

DAY	VOLUME	SP. G.	NITRO- GEN	CREATI- NINE	CREA- TINE	URIC ACID	AMMONIA- NITROGEN	P <sub>2</sub> O <sub>5</sub>	SODIUM SA- LICYLATE
	cc.		grams	grams	grams	grams	grams	grams	grams
June 7-8	1840	1.015	15.38	1.37	0	0.44	0.70	3.41	0
June 8-9	1400	1.019	14.20	1.36	0	0.35	0.70	3.50	0
June 9-10	1200	1.015	14.81	1.42	0	0.38	0.74	3.48	0
June 10-11									3.3
June 11-12	1180	1.020	15.2	1.33	0	0.64	0.56	3.63	5.0
June 12-13	1020	1.024	15.2	1.33	0	0.48	0.58	4.00	5.0
June 13-14	1120	1.023	15.3	1.35	0	0.42	0.80	4.12	5.0
June 14-15	1620	1.013	14.1	1.42	0	0.40	0.92	3.41	0
June 15-16	2250	1.009	11.2	1.33	0	0.40	0.69	2.42	0
June 16-17	1560	1.014	12.3	1.32	0	0.37	0.74	3.06	0
June 17-18	1020	1.025	14.0	1.40	0	0.36	0.62	3.23	0

TABLE II

*Basal metabolism determinations. Subject I, Mr. F. Height, 166 cm. Usual weight, 61 kg. Body surface, Du Bois Linear Formula 1.67 s.m.*

DATE	OXY- GEN CC. PER MIN.	CARBON DIOX- IDE CC. PER MIN.	R. Q.	CALOR- IES PER SQUARE METER PER HOUR	PULSE	TEMPERATURE, UPPER TRIANGLE— MORNING LOWER TRIANGLE— EVENING	SODIUM SALICYLATE
							grams
June 6.....	199	228			86	97.0	None
	177	203			87		
	186	237			87		
Average.....	187	223	0.84	38.9	87	97.8	
June 7.....	188	225			81	97.5	None
	182	221			80		
	188	234			82		
Average.....	186	227	0.82	39.4	81	98.0	
June 11.....	194	239			82	98.1	3.30 yesterday 0.65 before to- day's exper- iment.
	188	247			84		
	198	250			82		
Average.....	193	245	0.79	42.2	83	98.6	
June 12.....	210	252			84	97.8	5.00 yesterday 1.60 before to- day's exper- iment
	219	256			82		
	182	251			80		
Average.....	204	253	0.81	43.9	82	98.6	
June 13.....	234	276			79	97.7	5.00 yesterday 1.60 before to- day's exper- iment
	236	257			78		
	221	254			78		
Average.....	230	262	0.88	46.2	78	98.1	
June 14.....	199	254			81	97.8	5.00 yesterday None before today's ex- periment
	213	249			83		
	206	246			82		
Average.....	206	249	0.83	43.3	82	98.8	
June 16.....	198	241			83	98.0	None
	194	239			87		
	200	241			86		
Average.....	197	240	0.82	41.7	85	98.0	
June 18.....	204	240			90	98.0	None
	202	225			88		
	197	232			86		
Average.....	201	232	0.87	40.8	88	98.8	

When divided into periods the urinary data may be summarized as follows:

*Nitrogen*

Period I.....	44.39 grams or 14.79 grams per day
Period II.....	45.70 grams or 15.23 grams per day
Period III.....	51.60 grams or 12.90 grams per day

*Creatinine*

Period I.....	4.15 grams or 1.38 grams per day
Period II.....	4.01 grams or 1.34 grams per day
Period III.....	5.47 grams or 1.36 grams per day

*Uric acid*

Period I.....	1.17 grams or 0.39 grams per day
Period II.....	1.54 grams or 0.51 grams per day
Period III.....	1.57 grams or 0.39 grams per day

*Ammonia nitrogen*

Period I.....	2.14 grams or 0.71 grams per day
Period II.....	1.94 grams or 0.64 grams per day
Period III.....	2.97 grams or 0.74 grams per day

*P<sub>2</sub>O<sub>5</sub>*

Period I.....	10.39 grams or 3.46 grams per day
Period II.....	11.75 grams or 3.91 grams per day
Period III.....	12.12 grams or 3.03 grams per day

*Faeces*

*Nitrogen*

Period I.....	4.5 grams or 1.5 grams per day
Period II.....	5.5 grams or 1.3 grams per day
Period III.....	5.6 grams or 1.4 grams per day

Examination of the blood gave the following results:

June 7 (third day of fore period) non-protein nitrogen 28 mgm., uric acid 2.2 mgm. per 100 grams of blood.

June 14 (first day of after period) non-protein nitrogen 28.0 mgm., uric acid 0.4 mgm. per 100 grams of blood.

These results warrant little comment as it has recently been shown by Fine and Chase,<sup>9</sup> and by Denis<sup>10</sup> that the increased output of uric acid in the urine noted after the administration of

<sup>9</sup> Journ. Biol. Chem., xxi, p. 371 (1915).

<sup>10</sup> This Journal, vii, p. 255 (1915).



salicylates is due to a lowering of the kidney threshold for uric acid, resulting in a lowered concentration of this substance in the blood.

Mr. F. remained in good condition during the experiment but complained of a sensation of ringing in the ears during the last two days on which salicylates were administered.

As will be seen from the above results the administration of doses of sodium salicylate to subject I sufficient to produce toxic symptoms produces a slight increase in urinary nitrogen and phosphates, a marked increase (30 per cent) in uric acid, a decrease in the ammonia, and no change in the urinary creatinine or fecal nitrogen.

The basal metabolism of this subject, determined before the drug was given, was 39 calories per square meter per hour; this coincides exactly with the average for men of his age. While taking the salicylate there was a very definite rise. The maximum rise being 15 per cent above the normal, on June 13 when he had had 1.60 grams, an hour before the experiment. The rise was also apparent when he had had no salicylate later than the previous evening, as in the experiment of June 14, which shows a 10 per cent rise.

Subject II, Mr. L, a man 41 years old, weighing 73 kilos, was convalescing from a compound fracture of both bones of the lower leg which he had sustained about eight weeks before our experimental work was begun. The injury had been treated by bone plating. The daily ration of this man consisted of 200 grams of bread, 75 grams of butter, 2 eggs (selected to weigh 60 grams gross), 100 grams of lean beef, 25 grams of sugar, 150 grams of potato, 25 grams of wheat meal, 300 cc. of 20 per cent cream, 960 cc. of whole milk.

As in the case of our first subject quantities of bread, butter, beef, and wheat meal sufficient to last throughout the experiment were secured at the beginning of the work and kept in cold storage.

The experiment was divided into a fore period of six days (Period I); the urine and stools for the first two days of this period were discarded, only the excreta passed on the last four

days being analysed; a period of five days (Period II) during which sodium salicylate was administered, and an after period of four days (Period III).

In this case the daily urines were examined only for creatinine (in order to guard against any inaccuracy in collections). At the end of the experiment composite samples were made of the urines for the three periods, and these were analysed for nitrogen and for phosphates. The results obtained are given below.

TABLE III  
*Subject II, (Mr. L)*

	NITROGEN IN URINE	P <sub>2</sub> O <sub>5</sub> IN URINE	CREATINE IN URINE	NITROGEN IN FAECES	SODIUM SALICYLATE
	grams	grams	grams	grams	grams
Period I. (4 days)	35.2	11.82	6.16	5.84	0
Period II. (5 days).....	63.0	17.77	7.80	7.05	Day 1.....3.3 Day 2.....5.0 Day 3.....5.0 Day 4.....6.6 Day 5.....1.6
Period III. (4 days).....	48.8	12.53	6.19	5.48	0
	Average per day	Average per day	Average per day	Average per day	
Period I.....	8.8	2.95	1.54	1.46	
Period II.....	12.6	3.5	1.56	1.41	
Period III.....	12.2	3.1	1.54	1.34	

From the above results on subject II it will be seen that while there was produced during the salicylate period a very considerable increase in the nitrogen and phosphate metabolism which persisted into the after period there was little, if any, rise noted in the basal metabolism.

The basal metabolism of this subject, determined before salicylate administration was begun, was 45 calories per square meter an hour. This is some 15 per cent above the normal average. Mr. L, although a febrile, always had a rapid pulse, sweated freely and had a slight discharge from his wound. He was probably mildly septic and hence the high metabolism.

Subject III, Mr. B, 64 years old, weighing 57 kilos, was convalescing from a fracture of the femur sustained seven weeks before our experiment was started.

The diet of this subject consisted of 50 grams of bread, 100 grams of wheat flour, 2 eggs (selected to weigh 50 grams gross),

TABLE IV

*Basal metabolism determinations. Subject II. Mr. L. Height, 170 cm. Usual weight, 73 kg. Body surface, Du Bois, linear formula 1.74 s.m.*

DATE	OXY- GEN CC. PER MIN.	CARBON DIOX- IDE CC. PER MIN.	R. Q.	CALOR- IES PER SQUARE METER PER HOUR	PULSE	TEMPERATURE UPPER TRIANGLE— MORNING LOWER TRIANGLE— EVENING	SODIUM SALICYLATE
October 27.....	231	270			98	97.5	grams
	200	269			93		None
	213	281			93		
Average.....	215	273	0.79	45.0	95	98.2	
October 29.....	226	272			96	97.4	3.30 day before yesterday.
	215	267			96		5.00 yesterday
	225	285			96		
Average.....	222	275	0.81	45.5	96	98.3	None before today's ex- periment.
October 30.....	228	289			88	97.8	5.00 yesterday
	219	276			87		1.95 before to- day's exper- iment.
	225	260			86		
Average.....	224	275	0.815	45.5	87	98.5	
October 31.....	228	293			86	97.3	6.60 yesterday
	219	287			83		1.60 before to- day's exper- iment.
	216	283			84		
Average.....	221	288	0.77	47.2	84	97.8	

80 grams of butter, 100 grams of lean beef, 45 grams of sugar, 150 grams of potato, 120 cc. of 40 per cent cream, 330 cc. of whole milk.

The experiment (as in the case of subject II) was divided into a fore period (Period I) of six days during which only the excreta for the last four days was analysed, a period of five days during which sodium salicylate was administered (Period II) and after period of four days (Period III).

TABLE V  
Subject III, Mr. B.

DAY	VOLUME	NITROGEN	CREATININE	URIC ACID	P <sub>2</sub> O <sub>5</sub>	SODIUM SALICYLATE
	cc.	grams	grams	grams	grams	grams
3	1160	8.29	0.96	0.36	1.20	0
4	1240	6.15	0.95	0.35	1.36	0
5	1140	7.90	1.02	0.29	1.44	0
6	1200	8.35	0.92	0.30	1.55	0
7	1180	7.89	1.00	0.31	1.87	2.0
8	1200	9.18	1.08	0.34	1.68	4.0
9	1120	8.56	1.05	0.45	1.68	5.3
10	1560	9.67	1.15	0.58	2.18	6.6
11	1240	8.28	1.03	0.50	1.52	3.0
12	1420	9.04	0.91	0.30	1.59	0
13	1120	8.27	0.90	0.30	1.12	0
14	1220	10.21	0.90	0.32	1.10	0
15	1400	9.60	0.99	0.35	1.47	0

TABLE VI

Basal metabolism determinations. Subject III, Mr. B. Height, 1.67 cms. Usual weight, 57 kg. Body surface Du Bois "Height Weight" Formula, 1.63 s.m.

DATE	OXY-GEN CC. PER MIN.	CARBON DIOX-IDE CC. PER MIN.	R. Q.	CALORIES PER SQUARE METER PER HOUR	PULSE	TEMPERATURE UPPER TRIANGLE—MORNING LOWER TRIANGLE—EVENING	SODIUM SALICYLATE
February 28....	151 155 147	170 172 179			64 64 64	98.0	grams None
Average.....	151	174	0.87	31.3	64	98.4	
March 1.....	147 142 140	175 167 170			64 64 64	97.1	None
Average.....	143	171	0.84	30.5	64	98.3	
March 6.....	143 138	172 188			64 64	97.8	March 4, 2.00 grams.
Average.....	140	180	0.78	31.7	64	98.4	March 5, 4.00 grams. None before today's experiment.
March 8.....	167 167 133	215 194 184	0.78 0.86 0.72	37.8 34.8 31.9	70 68 64	97.6	March 6, 5.30 grams.
Average.....	156	198	0.79	34.8	67	98.0	March 7, 6.60 grams. Before today's experiment 1.30 grams.



When divided into periods the urinary data may be summarized as follows:

*Nitrogen*

.....	30.7 grams or 7.6 grams per day
.....	44.6 grams or 8.9 grams per day
.....	37.1 grams or 9.3 grams per day

*Creatinine*

Period I.....	3.85 grams or 0.96 grams per day
Period II.....	5.31 grams or 1.06 grams per day
Period III.....	3.70 grams or 0.92 grams per day

*Uric acid*

Period I.....	1.30 grams or 0.32 grams per day
Period II.....	2.18 grams or 0.43 grams per day
Period III.....	1.27 grams or 0.31 grams per day

$P_2O_5$

Period I.....	5.55 grams or 1.39 grams per day
Period II.....	8.93 grams or 1.78 grams per day
Period III.....	5.28 grams or 1.32 grams per day

*Fecal nitrogen*

Period I.....	3.64 grams or an average of 0.91 grams per day
Period II.....	3.92 grams or an average of 0.78 grams per day
Period III.....	3.70 grams or an average of 0.79 grams per day

In the case of Mr. B, as in the preceding experiments, there is a decided increase in the excretion of nitrogen, phosphates and uric acid, and in addition a slight increase in the creatinine output. The increased nitrogen output is also singularly persistent during the after period, a characteristic which is not shared by the other urinary constituents. The fecal nitrogen is somewhat diminished during the salicylate period and the after period a fact which was also noted by Wiley during his salicylate experiments and which was taken by him to indicate an increased absorption.

As in the case of Mr. L, however, the basal metabolism remained unchanged. His basal determined before the administration of the drug was begun was 31 calories, this is probably within 10 per cent of the average for his age. Like Mr. L this subject showed no symptoms of intoxication even on the day on which 6.6 grams of sodium salicylate was administered.

## CONCLUSIONS

Our results show in the case of two normal men that the administration of large doses of sodium salicylate (up to 6.6 grams per day) produces an increase in the excretion of nitrogen, phosphates, and uric acid. In one case, Mr. F, this increased nitrogenous metabolism was accompanied by an increase in the basal metabolism, and symptoms of salicylate intoxication (such as ringing in the ears). In the other case, Mr. B, a much greater increase in the urinary excretion of nitrogen (which extended throughout the after period) was observed, but there was no increase in the basal metabolism and no symptoms of intoxication. In one mildly septic individual, Mr. L, results similar to those secured with Mr. B were obtained. No change in the respiratory quotient occurred in any of these subjects.



## AN EXPLANATION OF THE LAXATIVE ACTION OF WHITE MUSTARD SEED

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In a paper on the laxative action of the seed of *Ocimum basilicum* (Malay: Sēlasih) and other viscous seeds<sup>1</sup> I remarked incidentally that this action, as regards white mustard seed, is not due to its mucus, but must be attributed to some other cause. I will now state my reasons for this view.

As I observed then, white mustard seed was mentioned long ago among the laxatives. William Cullen, the famous English physician (1712–1790), makes mention of this remedy in the following terms:<sup>2</sup>

A practice, so far as I can learn, first begun in this city (Edinburgh) fifty years ago, has been since very frequent. It consists in giving the mustard seed entire and unbruised to the quantity of half an ounce, or as much as an ordinary tablespoon will contain. This does not prove heating in the stomach, but stimulates the intestinal canal, and commonly proves laxative, or at least supports the usual daily excretion. It commonly also increases the secretion of urine; but in this I have found it frequently to fail. In giving it twice a day, as our common practice is, I have not found it to stimulate the system or heat the body; but it must certainly have that effect if it answers in the Swedish practice by giving it four or five times a day to prevent the recurrence of intermittent fevers.

For the rest not much has been written on the purgative qualities of mustard seed. The notice of Maccarten,<sup>3</sup> an Irishman resident at the time in Paris, on the therapeutic properties

<sup>1</sup> *Nederlandsch Tijdschrift voor Geneeskunde*.

<sup>2</sup> A treatise of the materia medica, MDCCLXXXIX, Vol. II, 171.

<sup>3</sup> *Journ. général de médecine*, 1809, t. xxxiv, 72.



of white mustard-seed is of no importance. A. Flückiger and D. Hanbury<sup>4</sup> have not even mentioned its laxative properties. Evidently they were known to Trousseau and Pidoux,<sup>5</sup> for these authors have recommended the seed against constipation, especially to sufferers from haemorrhoids; moreover—and this is remarkable experience, which in connection with what follows I wish to draw attention to—against eruptive skin affections and chronic rheumatism. Its salutary effect in these cases was attributed to the intestinal mucous membrane being stimulated, which, according to their pathological views, would result in “the blood being purified.”

I shall revert to this subject, but wish to point out first that mustard seed contains much less mucus than linseed, so that there are grounds for doubting whether the purgative effect of the two seeds is due to the same cause. This doubt is confirmed by the fact that, to bring about the result desired, two or three eggspoonfuls<sup>6</sup> of uncrushed white mustard seed are required, but of linseed as many tablespoonfuls; further by the circumstance that the use of linseed has never led to poisoning, which cannot be said of mustard seed. I do not mean those cases in which an accumulation of seeds sticking together leads to occlusion of the intestinal canal, for an accident like this, which may occur in a case of intestinal stenosis, will sometimes be occasioned by any kind of viscous seed, but an actual intoxication such as has been described by Kolbe.<sup>7</sup>

This mustard seed intoxication related to a woman who had been in the habit for a few years of taking now and again, every time three or four weeks running, a teaspoonful of mustard seed—it is not stated of what kind. This she did three times a day before meals, as a remedy against pains in the stomach. No grave consequences had been the result until one day, after the patient had been taking for a few days five or more teaspoonfuls,

<sup>4</sup> Pharmacographia, 2d ed., 1879.

<sup>5</sup> *Traité de Thérapeutique et de matière médicale*, 1785, 1, 607.

<sup>6</sup> An eggspoonful of white mustard seed weighs about 2.5 grammes, a tablespoonful about 14 grammes.

<sup>7</sup> Ueber Senfvergiftung. *Deutsche med. Woch.*, 1904, 237.

very serious intoxication symptoms manifested themselves, chiefly unconsciousness and cyanosis. Kobert, quoting this case in his Handbook<sup>8</sup> evidently did not think the cyanosis worthy of special mention, but it will presently appear that this symptom is of importance for the explanation of the mustard seed intoxication. Kolbe attributed the intoxication to allyl-mustard oil<sup>9</sup> and this explanation readily suggested itself since investigations of Mitscherlich<sup>10</sup> and Henze<sup>11</sup> had shown that volatile oils, allylmustard oil for instance, have a paralyzing effect upon the nervous system. Kolbe, however, has overlooked the fact that the cyanosis is not mentioned in these investigations.

I have asked myself how this cyanosis can be explained and I believe I have found an answer in the fact observed whilst investigating the viscosity of a mustard seed extract. I had left half a gramme of mustard seed in 100 cc. of water at a temperature of 37°C., and noticed the next day, contrary to all expectations, not the smell of mustard oil, but the unmistakable odor of *hydrogen sulphide*. This gas being a strong stimulus of intestinal movements, it seemed desirable to institute further researches, also as regards the extent of this gas-formation. For if this should turn out to be considerable, it would supply a more satisfactory explanation of the laxative action of mustard-seed than the one hitherto generally accepted.

It is easy to convince oneself of this H<sub>2</sub>S-formation; one need only leave some mustard seed, crushed or whole, in a closed bottle of water at room or body temperature. At this temperature it is great enough to be visible: on the seeds are found after some hours gas-beads, which consist, at least partly, of hydrogen sulphide, and when the process is in full activity, some foam even accumulates on the surface of the fluid.

In the case of white mustard seed only the smell of H<sub>2</sub>S is noticeable, but the black behaves somewhat differently. The

<sup>8</sup> Lehrbuch der Intoxikationen, 2e Aufl., 1906, ii, 36.

<sup>9</sup> Hence it may be inferred that his patient had been poisoned by black mustard seed.

<sup>10</sup> Ueber die Einwirkung der aetherischen Oele auf den thierischen Organismus. Preuss. med. Zeitung, 1843.

<sup>11</sup> Das aetherische Senföl. Diss. Halle, 1878.

latter develops, when crushed more than when uncrushed, the sharp smell characteristic of mustard oil, which is strong enough to hide the smell of  $\text{H}_2\text{S}$ . But the employment of chemical reagents shows that hydrogen sulphide really is formed.

It is absolutely certain that the gas evolved is indeed  $\text{H}_2\text{S}$ . It has the characteristic odor of that gas, colours an acetate of lead or a nitrate of silver paper, when held above the vessel, brownish black, and binds free iodine when led through a solution of iodine in potassium iodide.

The gas-formation may be prevented in various ways. First by great heat. Dry heat does less harm than moist heat; seeds which had been heated for an hour in a dry state at  $115^\circ\text{C}$ ., were found not to have altogether lost the power of forming  $\text{H}_2\text{S}$ , though it had evidently much decreased, for the gas could no longer be smelled. Boiling the seed for 10 minutes in water proved conclusive. Likewise a treatment during 24 hours with a 1 per cent formalin-solution. Other antiseptics, such as salicylic acid 1:500, sodium fluoride 1:100, a saturated solution of chloroform, a thick layer of toluene, or boiling for an hour in absolute alcohol were unable to prevent the process.

The  $\text{H}_2\text{S}$ -formation is by no means insignificant, as may be illustrated by the following experiment.

A certain amount of seed and water was exposed in a closed bottle to a temperature of  $37^\circ$ . To prevent bacterial fermentation the contents were covered with a thick layer of toluene. The bottle was provided with an ingoing tube, which opened in the fluid, and an outgoing one above it. The former was connected with a cylinder filled with compressed hydrogen, the latter with a battery of bottles filled with titrated fluids, which were to bind the gas-products. The hydrogen served partly to keep the oxygen out of the apparatus, so as to prevent decomposition of the hydrogen sulphide, partly to drive the gases formed, through the battery. To prevent loss of gas from leakage the positive pressure in the apparatus was kept as low as possible by a small supply of gas, and by means of a small waterdrop-pump.

The battery consisted of two parallel rows of washing-bottles which, with a view to the gas-determinations, could alternately



be put out of action without the need of interrupting the current of hydrogen. Each series consisted of five bottles, the first two of which were filled with titrated iodine-solution, the third with a potassium iodide solution, which served as an indicator to trace the loss of the volatile iodine, and two more, containing titrated  $\text{Ba}(\text{OH})_2$ -solutions which were destined for the determination of the carbon dioxide.

*Quantities of gas in milligrammes developed by:*

		1st 24 hrs.	2nd 24 hrs.	3rd 24 hrs.	4th 24 hrs.	5th 24 hrs.	6th 24 hrs.	7th 24 hrs.	8th 24 hrs.	9th 24 hrs.	10th 24 hrs.	11th 24 hrs.	12th 24 hrs.	AMOUNT IN 12 X 24 HOURS
25 grammes of white mus- tard seed	$\text{H}_2\text{S}$	1.02	1.36	1.19	1.02	0.85	0.68	0.34	0	—	—	—	—	6.46
	$\text{CO}_2$	0.6	35.1	29.5	25.5	23.7	6.4	4.1	2.6	0.9	0.9	0.4	0.1	129.8

This table shows that, besides  $\text{H}_2\text{S}$ ,  $\text{CO}_2$  is also formed and this formation is much more extensive than the former; that both processes, feeble at first, are marked by an increasing intensity, gradually decreasing after having reached an optimum, and that at last they cease altogether. The  $\text{H}_2\text{S}$ -development takes the lead at first. It already approaches the optimum on the first day, when the formation of  $\text{CO}_2$  is still relatively small, but the  $\text{CO}_2$ -formation soon surpasses it.

The impeding effect of heat and formalin, both powerful anti-fermentative agents, on the  $\text{H}_2\text{S}$ -formation, naturally suggests some connection with ferment-action.

In order to gain an insight into what takes place in the mustard-seed under the influence of moderate heat and moisture it is necessary to take its component parts into account.

Its characteristic elements are:

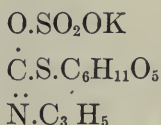
1. Fat oil, found in the cells of the endosperm and of the embryo. The black mustard contains from 15–20 per cent, the white 25–35 per cent and the Indian or Sarepta mustard (from *Sinapsis juncea* L.) about 30 per cent. The greater part of this oil may be removed by pressing the seed. The rest is an article of commerce known as mustard-cake or mustard powder.



2. The glucoside-splitting ferment myrosin found by Guignard<sup>12</sup> in separate dispersed cells, the properties of which ferment have been further investigated by W. Spatzier.<sup>13</sup>

3. The glucoside sinigrin (a potassium salt of myronic acid) in black mustard, and

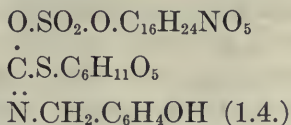
4. the glucoside sinalbin in white mustard. Sinigrin is according to Gadamer composed as follows:



It breaks down whilst taking up water into allyl-mustard-oil,<sup>14</sup> d-glucose and potassium hydrogen sulphate:



Sinalbin is, as Gadamer writes, composed as follows:



When acted upon by myrosin, it gives p-oxybenzylmustard-oil, d-glucose and sinapin sulphate.



The white mustard-oil, with which we are chiefly occupied, is insoluble in water. It tastes hot, and like allylmustard-oil it produces blisters on the skin, but not so quickly. It has no pungent smell and is indeed generally looked upon as fixed. This will be one of the reasons why white mustard-meal, from which the fat has not been removed, gives, on being mixed with water, no mustard-smell as black mustard does. But also the fat, so

<sup>12</sup> Sur la localisation des principes qui fournissent les essences sulfurées des crucifères. *Compt. rend. de l'Acad. d. Sc. T. III.* 249, 920.

<sup>13</sup> Ueber das Auftreten und die physiologische Bedeutung des Myrosins in der Pflanze. *Pringsheim's Jahrb. f. wiss. Botanik*, 1893, Bd. 25, 39.

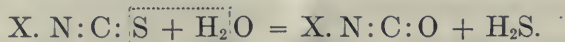
<sup>14</sup> This oil was first isolated by Boerhaave. *Elem. Chem.*, Process xx and xxxiii.

abundant in the white seed, has an impeding effect on the reaction.

On account of these facts it may already be pointed out that for certain therapeutic purposes it is not indifferent whether the white or the black seed is used. The black seed, containing less fat, will, on account of the pungent oil, which is easily set free, be less suitable for internal use than the white, unless as an emetic, for which it was formerly particularly suited on that very ground. On the other hand it is to be preferred as a skin-stimulant, when bruised of course, and deprived of its fat. Now it also becomes clear why for laxative purposes the white seed has come to be preferred to the black. I may here observe that in the literature we find merely mentioned that mustard-meal mixed with water gives mustard-oil. The handbooks might add that, as regards white mustard, meal deprived of its fat should be recommended. The experiment may fail if made with crushed seeds from which the fat has not been removed. The reason why this has not been noticed before is that for the experiment mustard-meal is used as supplied by the shops, from which the fat has already been extracted.

The impeding effect of heat and of the anti-fermentative formalin suggests that the  $\text{H}_2\text{S}$ -development is connected with the decomposition of sinigrin and sinalbin. The  $\text{H}_2\text{S}$  set free may be accounted for by assuming that at the decomposition of the glucoside a thio-derivative is formed which, with  $\text{H}_2\text{O}$ , results in  $\text{H}_2\text{S}$ , or that the thiocyanic group of the mustard-oil first formed is decomposed while uniting with water. A glance at the constitutional formulae of the black and the white mustard-oil teaches that the gas can only originate from the thiocyanic group.

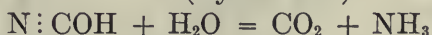
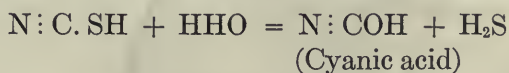
The nitrogen and the sulphur of the thiocyanic group are both unsaturated, which of course promotes the absorption of water and its decomposition. This process may be stated as follows:



The isocyanate formed is decomposed with absorption of water as follows:



It is a remarkable fact that J. Gadamer<sup>15</sup> who has carried out an extensive investigation of the component parts of mustard-seed, has not noticed the hydrogen sulphide. Being engaged upon an examination of white mustard (in which König supposed that sinigrin was to be found) he observed that when a mixture of it was distilled with water (which mixture, he writes, did *not* smell of mustard-oil), a coating of sulphur had settled in the refrigerator, and also that the ammonia-solution, in which the distillate was received, turned yellow. In this solution nitrate of silver produced a thick precipitate of silver sulphide, whence Gadamer did not think it impossible, that thiosinamin had been formed. But, he continues, the yellow colour disappeared entirely when the distillate was evaporated, whilst at the same time sulphur was set free, the solution giving at last but a faint precipitate with nitras argenti; hence he concluded that the yellow colour must be due to ammonium sulphide, which may have been formed by the distilled sulphur being dissolved in the ammonia. The origin of this sulphur he traced to sulphocyanic acid which may have been formed when the unstable sinalbin-mustard-oil was heated and which may have been distilled with the water-vapour. If, then, all this is correct, the formation of H<sub>2</sub>S and CO<sub>2</sub> can be accounted for. It may take place as follows:



It is difficult to account for this great difference between the volumes of H<sub>2</sub>S and CO<sub>2</sub>. The glucose group might be viewed as the origin of the CO<sub>2</sub>, but not necessarily so. The difference might very well be due to the greater solubility of H<sub>2</sub>S in water, which is almost three times as great as that of CO<sub>2</sub>; but part of the gas may also combine with amines set free. In this case the difference would only be a seeming one.

After this digression, justifiable from a scientific point of view, on the origin of hydrogen sulphide and the degree in which it is

<sup>15</sup> Ueber die Bestandteile des schwarzen und des weissen Senfsamens. Arch. f. Pharmazie, Bd. 235, 1897, 44.



formed, we may return to the laxative action of the white mustard-seed, and ask ourselves first whether we may put down this effect to the  $H_2S$ . The peristaltic properties of  $H_2S$  are well known. On it is based the action of sulphur, the laxative especially applied in the case of haemorrhoids, and much too little appreciated as such, its only drawback being the flatulence which it causes. From the usual doses of 2 or 3 grammes it might be inferred that it requires considerable quantities of  $H_2S$  to strengthen the peristalsis. This, however, is not the case. The sulphur is reduced so slowly in the intestine that the greater part is not affected by the process, and leaves the body unchanged. The activity of the gas should, therefore, not be underrated. This might already be concluded from its extreme poisonousness. It is true the quantities found in vitro, in our quantitative determinations, are slight, but there are grounds for assuming that in reality they will be much greater. For the method has of course its defects, one of which is found in the instability of the gas. Hence the divergent results of my determinations. In another experiment 25 grammes of seed had developed 7.85 mgm. of  $H_2S$  in 3 days; another time one gramme of mustard seed, which had been left for 18 hours at  $37^\circ C$ . in a litre of water, gave 2.1 mgm. One should, however, be very circumspect as regards the mucus which was found to stimulate the decomposition. The pale yellow colour which the water above the seeds assumes points to the presence of free sulphur and the nitroprusside sodium-reaction confirms this. What is more, I found that in the air above the seeds, which had been deprived of the greater part of their slime by being shaken with water and ground glass at  $0^\circ C$ . greater quantities of hydrogen sulphide were to be found than above seeds which had been left in a slime-infusion. In the intestine this decomposition will take place in a less marked degree because the slime, owing to the continual peristalsis, and its property of passing easily into water<sup>16</sup> will have been dissolved for the greater part, by the time the seed has reached the large colon intestine, and will have been digested in its diluted state. It may be objected

<sup>16</sup> Nederlandsch Tijdschrift voor Geneeskunde. 1916.



that it takes several days to develop some milligrammes, whilst the seed is sure to remain no longer than a day in the intestinal canal, but then the  $H_2S$ -formation soon reaches its maximum. The determinations give us a right to assume that a dose of 10 grammes of seed (3 eggspoons) can produce on the first day at least from 0.5 to 1 mgm. of  $H_2S$ . This gives, the contents of the large intestine being, at a liberal estimate, 2 kg., a concentration of 1:4,000,000. How active the gas may be in this concentration appears from the subjoined curve, showing its effect on

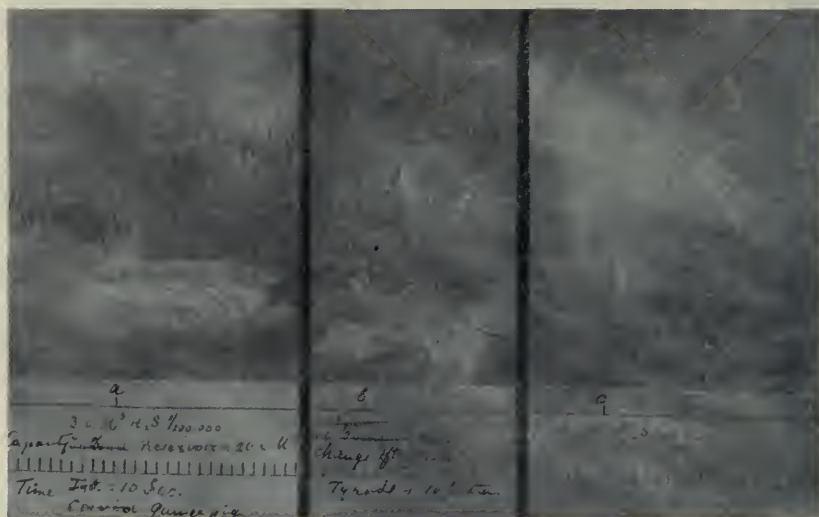


FIG. 1 ISOLATED LOOP OF INTESTINE OF GUINEA-PIG IN TYRODE'S SOLUTION

Suspension method. Upstroke=contraction. Time 10 minutes. At C change to fresh Tyrode's solution. Capacity of reservoir 20 cc.

the isolated loop of intestine of a guinea pig where it effects a considerable increase of the tonus.

Finally I may remark that the carbon dioxide also has its share in the increased peristalsis. Of this gas, which is known to be a strong stimulant, considerable quantities are present.

The reader will have guessed why I pointed out that Kolbe did not pay a sufficient amount of attention to the cyanosis of his patient. Undoubtedly it was a symptom of sulphhaemoglo-

binaemia. It must be remembered that the patient had taken great quantities of mustard-seed and that consequently much  $H_2S$  must have been formed. It is difficult to say why, in this special case, it should have accumulated in the blood. But we know how unreliable the organism is in the matter of resorption, or rather, the conditions, governing it, are not always known to us. The fact that the patient had taken black mustard seed, is of no importance, for the black produces  $H_2S$  as well as the white. To what extent I have not been able to determine quantitatively, amongst other reasons because the iodometric determination in such a case as the one under consideration, where we have to deal with a volatile allyl-compound, offers difficulties, the solution of which would, for the present, have led me too far from my subject. The reaction with the lead acetate-paper, however, shows that the  $H_2S$ -formation takes place on at least as large a scale. In this connection I may remind of the cyanosis which Stokvis<sup>17</sup> has described as "enterogeneous" and which, in a case that has been carefully investigated by Hijmans van den Berg<sup>18</sup> must undoubtedly be attributed to the presence of sulph-haemoglobin, caused by  $H_2S$  which had been absorbed from the intestine.

The reason why sulphur or other substances producing  $H_2S$  do not oftener cause cyanosis and other symptoms of intoxication, finds its explanation in an instructive experiment by Claude Bernard,<sup>19</sup> which Stokvis used to carry out in a somewhat modified form at his interesting lectures.<sup>20</sup> Injection in the rectum or, what is more, in the vena jugularis of some animal may be carried out without any injurious effect, if only care is taken that not too much is injected at one time. The brown colour of a test-paper held before the mouth shows how quickly and by which way the poison leaves the body. As long as the excretion by the respiratory canal keeps pace with the absorption, the

<sup>17</sup> Nederlandsch Tijdschrift voor Geneeskunde, 1902, ii, 678.

<sup>18</sup> Ned. Tijdschr. v. Geneesk. 1905, i, 719.

<sup>19</sup> Leçons sur les effets des substances toxiques et médicamenteuses, Paris, 1857, 57.

<sup>20</sup> Voorhrachten over geneesmiddelleer, 3d Ed., revised by Dr. H. Zeehuisen, 1906, i, 93.

animal will not be affected, but as soon as something goes wrong with this mechanism, the intoxication-symptoms appear.

One more remark. As we know sulphur and sulphur-springs have been applied since time immemorial in cases of eruptive skin-diseases and rheumatism. The effect of these remedies, which still hold their ground, is doubted by some pharmacologists because the scientific proof is wanting. But have we a right to refuse on such grounds credence to a time-honoured experience? Now that we have become somewhat better acquainted with the property of mustard-seed of producing  $H_2S$ , we are led to think of its old application against eczema and rheumatism, as we are told by Trousseau and Pidoux. If we, who are scientifically trained, fail to discover the connection between the properties of remedies and their therapeutic application, we are sometimes a little too much inclined to doubt their rationality. The white mustard-seed may teach a lesson and contribute to heighten our respect for that practical insight of our predecessors, which was their only but very sharp weapon.

## SOME REACTIONS OF BLOOD VESSELS TO CERTAIN CHEMICALS

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The various tissues and organs of the body receive from the circulating blood such material as they require for their nutrition and function; they, on the other hand, must necessarily discharge some of the products of their metabolism into the interstitial tissue and its lymph spaces. The blood vessels are therefore likely to be steeped in media more or less unstable in their chemical composition, and varying widely in different regions of the body; and it is to be assumed a priori that the vascular system reacts in certain definite ways to the physical and chemical changes that are more or less continuously taking place round about it. The study of the effects of chemical reagents, of the products of glandular secretion, of the action of drugs, upon the heart and the blood vessels, especially upon the arteries has been mainly carried out by methods of perfusion. The direct observation of the living vessels under varying mechanical, thermal, electrical, and chemical conditions, has received comparatively little attention. As early as 1852, H. Weber (43) published some experiments on the web of the frog's foot. In those ante-Cohnheim days the various theories of inflammation were much concerned with stasis in the arteries, veins, and capillaries, as a fundamental factor in inflammatory processes. Weber's experiments were undertaken from this point of view, but he mentions rather incidentally, that dilute potassium and ammonium hydrate cause contraction of the artery, but not (as he erroneously states), of the vein. He has also observed the back flow of the blood from the veins into the capillaries under



certain abnormal conditions. The brilliant researches of Cohnheim (6, 7, 8) focussed the interest of the entire scientific world for a time upon the tongue and mesentery of the living frog, but mainly for the study of inflammation and the many new and far reaching problems arising therefrom. W. H. Gaskell (10) in 1880-1882, in his articles on the Tonicity of the Heart and the Blood Vessels made use principally of perfusion, but he records also some observations upon the direct influence of certain chemicals upon the frog's mesentery. He states that solutions of sodium hydrate of 1-10,000 or even 1-20,000 contract blood vessels and that the contraction may lead to absolute closure. Lactic acid on the other hand, 1-10,000 or 1-13,000 always dilates the vessels. In proof of this he gives some measurements, based however on perfusions, and generalizes that alkalies tend to contract, acids tend to dilate the blood vessels. Recognizing the great similarity which in many respects exists between the physiological properties of the heart muscle and the smooth muscle of the arteries and veins, he develops at some length a very fascinating and plausible theory of the tonicity of the cardio-vascular system in which a dominant part is assigned to chemical control. More recently Natus (33) published a most careful study of the effects of various kinds of stimulation, principally thermal and chemical, upon the blood vessels of the mesentery and of the pancreas of living rabbits. This work was evidently designed as a basis for a theoretical discussion of stasis and its relations to the theory of inflammation, with a strong inclination in favor of nerve control as a fundamental factor in all these processes. It contains however an abundance of valuable observations to which frequent reference will have to be made. It is but fair to state that the present writer had no knowledge of the work of Gaskell and of Natus until a few months ago, when his own work was already advanced. While engaged in the study of certain problems in the pathology of arteries, it was thought useful to investigate some of the reactions of living normal vessels, especially of arteries, towards certain chemicals, not by the indirect methods of perfusion, but by direct observation under the microscope. These observations were carried on

for some length of time entirely independent of the work of others.

The great majority of these experiments were done on the frog. The frog's tongue is not a favorable object, for the massive layers of epithelium and the profuse secretion of mucus make the contact of the reagents with the blood vessels very uncertain. Alkali seemed to reach the blood vessels somewhat more readily, but acids were probably largely detained by the mucus. (Pawlow estimates that the mucus of the stomach neutralizes up to 25 per cent of its acidity, and one factor in the etiology of round ulcer of the stomach may possibly be, as Kaufmann has suggested, an insufficient secretion of mucus.) The mesentery therefore, was the object mainly employed. The frogs were either decerebrated and pithed, or curarized, and it may be stated at once that no essential difference in the reactions was recognizable, whether the central nervous system was destroyed or whether the animal was simply put under the influence of curare. The mesentery was spread over a small cork ring in the usual manner, but the spontaneous rhythmical motions transmitted to the entire mesentery from the intestines were often annoying, as they were liable to interfere with the accuracy of the measurements. The small arteries, and more still the veins, are highly sensitive to overstretching, and respond to the slightest strain beyond their normal adjustments with distortions and contractions. Complete stoppage of the circulation can be caused by incautious stretching, and when that happens it is as a rule impossible to restore the blood current even after all tension is eliminated. Resting the mesentery upon a cover glass proved no help, and was inconvenient in many other ways. All reagents were dissolved in 0.75 per cent saline. It was necessary to guard carefully against piling an excessive quantity of fluid upon the surface of the mesentery, for the mere weight of the liquid would often be sufficient to overstretch the vessels and cause serious interference with the blood current. The solutions were therefore applied by means of a small glass tube having a rubber bulb at one end and the other drawn out into a moderately fine capillary. A delicate thread of silk or yarn was drawn through the

tube, fastened at the bulbar end and allowed to protrude for the length of a millimeter or two beyond the capillary opening. With this little tube, and with the aid of an ordinary dissecting microscope, it was easy to secure direct contact with whatever part of an artery or vein one might select, and to apply the reagent in any desirable quantity. The thread, though thoroughly saturated with fluid, would nevertheless frequently cling to the vessels, and the latter, and with them often the entire mesentery, would be apt to be rather roughly dragged about. Under normal conditions and with good circulation the purely mechanical irritants that could be caused by the fine thread would have no apparent effect. It was often necessary, too, to cleanse the surface of the mesentery with some absorbent material, usually filter or litmus paper. This also might involve considerable mechanical irritation, but under ordinary circumstances would cause no disturbance. Under abnormal conditions however, even slight contact with the thread or a very gentle application of filter paper might cause vascular constriction, first always, and most readily in the smallest veins, then in the larger ones, and then in the smaller arteries, but never, in our observation, in the large main trunks at the root of the mesentery. With the magnification employed in all these experiments, nothing very definite can be said about the behavior of the capillaries. The impression was that they were not affected. Now and then a lively current might be noticed in a group of capillaries, while the arterioles in the immediate vicinity were closely contracted or showed little or no current. The reverse also was not infrequently observed, namely that the current in the capillaries ceased, while the arteries and veins were apparently quite normal. The deformation caused by purely mechanical agencies, if not too severe, and if conditions are otherwise favorable, right themselves, as a rule spontaneously and within a reasonably short time. The upper limit to which this mechanical interference can be carried without causing serious damage probably varies in different organs and also with the structure of the vessels and with the blood pressure, (Straub (38), Winkler (44). All observations were made with a Zeiss Planar lens which mag-



nified sufficiently for the demonstration of all necessary details, and having a focal distance of 20 mm., permitted all desired manipulations on the mesentery. In addition to this, a Zeiss No. 6 compensating ocular was used which contained a micrometer scale, each division of which, in this combination, was equal to about 0.025 mm. An ordinary dissecting microscope was also found useful.

#### ALKALIES

All alkalies tend to contract the blood vessels (let it be again understood that capillaries are not considered, except when special mention is made of them). Up to a certain limit of dilution, there is no reaction. Beyond this limit the contraction of the vessels is in proportion to the concentration. The immediate effect of the contact of the alkaline fluid with the blood vessels is usually a stoppage of the current in the arteries as well as in the veins, often with reversal of the direction of current, especially in the arteries, and oscillation of the blood column to and fro. Cohnheim has already mentioned this "*va et vient*" current. Oliver (34) and others have also taken note of this occasional cessation and reversal of the blood stream. With concentrations just strong enough to cause a mild reaction, the current recovers almost immediately and continues regularly and in its proper direction, though nearly always slower than normal, and now constrictions begin to appear. There is no evidence of any specific point of attack. The smaller veins and arteries are most easily affected, but the action of the alkali may be promptly recognized wherever the solution happens to come into contact with the vessels. By means of the capillary tube and string, it is sometimes possible to confine the action of the reagent to a single little spot on a vessel, and in that case a constriction will appear in that place only (fig. 1). This strict localization is however not often possible. The surface of the mesentery is never quite level nor quite dry. There is also more or less continuous motion, and therefore there will always be some diffusion, often in quite unexpected directions and over wide areas.



Braeuning (5), who experimented on the web of the frog's foot, has already pointed out the importance of diffusion in determining the extent to which the vascular apparatus will be affected. With weak solutions the narrowing of the vascular lumen proceeds very gradually. No definite peristaltic wave is ever to be recognized, and the direction in which the contraction advances is determined, apparently only by the laws of diffusion. If the concentration is just strong enough to produce a moderate



FIG. 1. A group of vessels at the root of mesentery. An isolated constriction of a vein touched with  $\text{KOH } \frac{\text{mol}}{20}$ .

effect, the contractions gradually increase up to a certain limit, but never to absolute closure, and beyond this limit the process remains stationary for some time. At this stage there appear almost regularly certain asymmetries in the outlines of the vessels. These have been noticed already by some observers, especially by Natus. The vessel is not uniformly contracted but presents a series of more or less close constrictions alternating with wider areas. In well defined cases this suggests a faint

similarity with beads on a string, and hence, for want of a better word, this peculiar phenomena is referred to in our protocols as "beading." On closer scrutiny of this so-called beading, one sees that the vascular walls are not smooth and even, but that there are areas of thickening, sometimes on one side only, more often on both sides of the wall and more or less regularly distributed along the vessels, opposite to each other. These areas of increased thickness correspond to the places of constriction, and the degree of thickening is in direct proportion to the degree of contraction. In the more advanced stages of contraction, the thickening of the walls of the vessels will, as has been stated, be uniform and perfectly even throughout the entire length of the contracted portion. It is only in the lesser degrees of shrinkage that these partial strictures appear, the mechanism of which is not quite clear. It is difficult to understand why a few circular fibers are thus seen to contract and others immediately adjoining on both sides preserve their normal tonus. It does not make any difference whether the central nervous system is destroyed or whether the animal is merely curarized.

Under the influence of the alkali the blood current as a rule is slower than normal, but it not infrequently happens, and for reasons that are not quite clear, that the current races with great velocity through the contracted vessels. With increasing concentrations the contractions become closer, so that only an extremely thin stream of plasma with cells apparently in single file is seen to pass through the arteries, while in the irregularly contracted vein the current has entirely ceased, or is extremely sluggish and oscillating jerkily backward and forward (fig. 2). It can thus come about that all the vessels, arteries, veins and capillaries of the exposed mesentery are finally collapsed and, with the exception of the main trunks at the root, completely empty or nearly so; possibly some plasma carrying an occasional blood corpuscle still trickling through here and there. If this condition is not permitted to last too long, and the concentration of the alkali has not been strong enough to directly injure the vascular apparatus, complete restitution to the normal is still possible. By removing the alkali from the surface of the

mesentery with filter paper and careful washing with normal saline, the normal circulation may gradually be restored, and it is surprising to see, when the circulation seems hopelessly gone, how it will sometimes pick up again and in course of time be as vigorous as ever. This restitution to the normal can also take

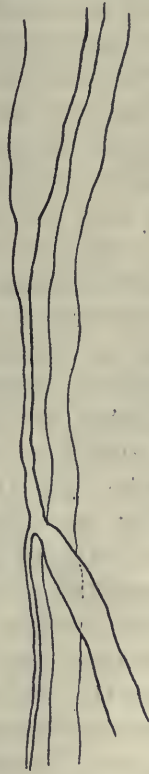


FIG. 2. Shows extreme contraction of artery and in less degree of vein, with some "beading," after application of  $\text{KOH} \frac{\text{mol}}{80}$ .

place spontaneously without any extraneous effort to remove the alkali, presumably when the quantity of alkaline solution and its concentration are not too great, and the normal defenses of the tissues are sufficient to cope with it. The application of stronger alkaline solutions is followed almost immediately by very close contraction of arteries and veins and complete stop-



page of the current. The vessels are empty and narrowed down to a thin thread. The vascular walls stand out quite distinctly, are apparently thicker than normal and quite even without any trace of "beading." When this stage has been reached, no recovery is possible. All efforts to neutralize the alkali by means of dilute acids or to remove it by the usual methods of absorption and washing, are of no avail, and the circulation remains irretrievably abolished. When weaker concentrations are employed, alternation of more or less close constriction and return to the normal may be repeated several times. It is to be noted, however, as Braeuning has already pointed out, that the irritability of the vascular muscle increases with each successive stimulation, and that there is thus a summation of effects; so that after a certain time, the limits of which have not yet been ascertained, the alternation of contraction and relaxation can no longer be produced and the vessels finally remain in a state of permanent contraction. Natus finds that the veins are less susceptible and respond more slowly to irritation than the arteries. In our own experiments much irregularity in this respect could be observed. Sometimes it was the vein which responded almost instantaneously to the alkali, while the artery remained unaffected for a much longer time. At other times the arteries could be seen contracted almost to complete closure, while the veins, though the current in them was sluggish almost to stasis, showed no signs of contraction. There evidently are a number of highly complex factors, leaving out of consideration altogether the functions of the nerves, which are concerned in these processes. It is probably safe to conclude that there is no difference in principle regarding the response to the action of alkalies between arteries and veins.

Nothnagel (32) in a series of well known experiments found that potassium affects the smooth muscle of the intestine of the rabbit otherwise than sodium, and he suggested that potassium probably acts directly upon the muscle, while sodium produces its effect by means of stimulation of the constrictor nerves. In our experiments no difference in the action of hydrate of potassium, sodium and ammonium upon the smooth muscle of the



blood vessels could be seen. The constricting effect, its intensity and form, appeared to depend entirely upon the hydroxyl-concentration. The lower limit of this, that is the concentration at which the very first and just recognizable, but momentary, constricting effect is visible and which often requires some additional mechanical stimulation, is in the neighborhood of  $\text{pH} = 9$ . The behavior of  $\text{NaHCO}_3$  may serve as an illustration. Concentrations of  $\frac{\text{mol}}{20}$  and even  $\frac{\text{mol}}{10}$  distinctly alkaline to litmus cause but a very slight narrowing and beading of the veins and to a less degree of the arteries. Very frequently this constricting effect becomes apparent only after the surface of the mesentery has been slightly compressed with litmus paper for the purpose of drying and of testing the reaction. This purely mechanical irritation, which as numerous experiments have shown, does not cause any disturbance under normal conditions, will now under the influence of the bicarbonate of soda almost invariably cause a number of constrictions to appear. It is quite possible that the pressure of the litmus paper serves to bring the alkaline fluid into closer touch with the blood vessels and thus promotes the constricting effect. It should also be considered that possibly carbonic acid may be set free and that this would tend to counteract the effect of alkali. The quite unusual dark color of the blood circulating in the veins, causing them to appear almost black, whenever  $\text{NaHCO}_3$  is applied, seems to speak for this, but as the hydroxyl-concentration of the solution lies between  $\text{pH} = 8.5$  and  $\text{pH} = 9$ , this fact is in itself sufficient to account for the uncertain action of the sodium bicarbonate. A concentration of about  $\text{pH} = 11$  seems to be necessary to induce constant, but very mild response, while forceful and close constrictions follow immediately upon concentrations of  $\text{pH} = 12$ . Our experience, as will be readily seen from this, differs from that of Hooker (19, 20), who concludes from his perfusion experiments that vascular tone is diminished by potassium and sodium ions.

## ACIDS

The action of acids upon blood vessels is very much more complicated than that of alkalies. All observers seem to agree that dilute acids stimulate the blood current and dilate the blood vessels, principally the arteries, but also the veins, and probably also the capillaries (Severini (37)). The peculiar arrangement of the musculature of the blood vessels admits of contraction even to complete closure, but there appears to be no special apparatus for active dilatation, so that any increase in diameter is brought about by relaxation of the contracting fibers and cannot therefore greatly exceed the normal. While the constricting effect of alkalies is fairly constant and uniform, there is not quite so much uniformity in the action of acids. Loeb (24) has long ago called attention to the fundamental differences in the biological action of various kinds of acids. On the whole it may be said of acids just as well as of alkalies, that their action upon blood vessels depends largely upon their hydrogen-ion concentration. Concentrations of about  $\text{pH} = 3$  seem to be approximately the lower limit of dilator activity. As the concentrations progress towards the neutral limit, they become inactive, but concentrations much below  $\text{pH} = 3$  seem invariably to cause *constriction*. It is not the purpose of the present writer to attempt an analysis of these extremely complicated chemical and physical conditions and of the disturbances of what has very recently been designated as the Vital Equilibrium (Spaeth (40)). It is merely proposed to report some observations without any attempt at elaborate interpretation.

The dilating effect of very dilute acids is as a rule rather transitory, and the return to normal dimensions occurs quite promptly, as soon as the acid stimulation is interrupted; this return taking place much more quickly than the recovery after even very slight alkaline reactions. Some acids retain their dilating properties in dilutions that no longer redden litmus. It may sometimes be observed that solutions rendering the surface of the mesentery distinctly acid to litmus, will no longer show this reaction when their stimulating effect upon the circulation has

passed, and the vessels have returned to the normal. This may at times perhaps, be due to the inadvertant contamination of the surface of the mesentery with intestinal contents or with the frequently quite profuse abdominal fluid. In the case of certain acids, however, as for example acid sodium phosphate, lactic acid, and others, the tissues themselves may be supposed in all likelihood to take an active part in this neutralization. Application of stronger solutions may perhaps be followed by an instantaneous but only momentary stimulation of the current and

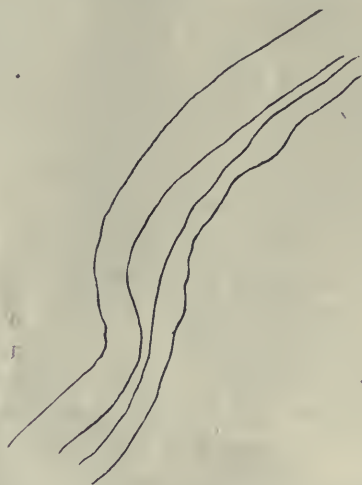


FIG. 3. Small artery and vein both showing considerable constriction and "beading" after application of  $\text{HCl } \frac{\text{mol}}{80}$ . The smaller vessel is the artery.

a dilatation almost too evanescent to be measured, but to this succeeds without exception, a more or less complete stoppage of the blood current and *constriction* of the vessels. The same uncertainty as regards the relative susceptibility of arteries and veins, that has been noticed with alkalies, obtains also with acids. Sometimes the veins are apparently more easily affected, and again the arteries seem more liable to constriction. Dilatation is certainly more pronounced in the arteries. Altogether the impression remains that the arteries respond more promptly and vigorously (fig. 3). The contractions of the vessels are



rarely as extensive as in the case of strong alkali, but are usually more or less localized, due to irregular conditions of diffusion. In further contrast to alkaline reaction, the blood vessels are not emptied and collapsed, but the current seems to stop abruptly, leaving the vessels, except in those places where there is very close constriction, filled with blood, often clotting, but not showing any signs of hemolysis. These results seem to indicate that the muscles of the blood vessels react somewhat differently to acids and alkali than the heart muscle, for Löevenhart and Eyster (26) find that the heart when perfused with alkali, contracts, but comes to rest in relaxation, while perfusion with acid dilates it, but terminates in contraction. Natus concludes that the vasodilators respond more promptly to weak stimuli, but that the vaso-constrictor effect predominates as the stimulation becomes more potent. He has not undertaken a systematic investigation of the action of acids and alkalies, but it is interesting to note that our results with acids upon the mesentery of the frog are almost identical with the effect observed by him of increasingly high temperatures upon the mesentery and pancreas of the rabbit. Dilute acids do not as a rule act as promptly as alkali. It seems occasionally as if a certain time were required for the acid to unfold its activity, though this varies very much with the nature of the acid and the degree of concentration.

Butyric acid for instance, in adequate concentration, say  $\frac{\text{mol}}{20}$

pH = 3, dilates and stimulates instantaneously.<sup>1</sup> Hydrochloric

acid  $\frac{\text{mol}}{1000}$  pH = 3, acts so, that an artery which may have meas-

ured 6 (these figures indicate the number of spaces of the micrometer scale), is presently, after the application of the acid, dilated up to 8, and the blood current is seen rushing through the

<sup>1</sup> One peculiarity of this acid ought not to pass without notice. In a certain unexplained way the frogs seemed to be extremely sensitive to this acid. It seemed to make no difference whether they were merely under the influence of curare or whether the brain and spinal cord had been completely destroyed. At each application of the acid, the animal would twitch violently, though making no attempt to wipe away the irritant with its feet.



vessels with considerably increased velocity. The application, however of  $\frac{\text{mol}}{100}$  pH = 2,  $\frac{\text{mol}}{20}$  pH = 1.4,  $\frac{\text{mol}}{10}$  pH = 1.1 cause progressively greater constrictions and very soon complete suspension of the circulation. Sulphuric and nitric acid behave in all essentials like hydrochloric. Boric acid, on the other hand, in a dilution of  $\frac{\text{mol}}{100}$  pH = 5.4 will dilate an artery 5 to 6. In much the same way have tartaric, phosphoric, acetic, oxalic, benzoic, formic acids, and acid phosphate of soda been found to behave. Lactic acid follows approximately the same rules  $\frac{\text{mol}}{20}$  pH = 2.6 can still dilate an artery from 4 to 6; concentrations below this however, act as vaso-constrictors.

A number of neutral salts proved entirely inactive. Thus magnesium sulphate and manganous sulphate in  $\frac{\text{mol}}{10}$  dilution had no effect whatsoever. Hypertonic (2 per cent) NaCl solution had a very curious action. A few drops upon the mesentery caused all circulation to stop immediately. It seemed as if there remained no time for a single pulsation, and arteries, veins and capillaries, appeared filled to the utmost with blood and the picture which the mesentery presented was very much like that of an artificial injection. Calcium chloride in thin dilutions had no effect. A moderate constricting action became evident at about  $\frac{\text{mol}}{20}$ , but only after a period of latency varying between 4 and 10 minutes and even then the contractions developed very slowly and gradually. Barium chloride acts as a very powerful constricting in dilutions of about  $\frac{\text{mol}}{30}$ , the constricting action increasing in intensity with higher concentrations.  $\frac{\text{Mol}}{100}$  has no effect. It takes as a rule fully 5 minutes before the constricting effect of barium becomes visible. Lead acetate and nitrate, in dilutions of  $\frac{\text{mol}}{100}$  and even less, cause prompt and intense con-

traction and stoppage of the blood current. It is probable that the constricting effect of these, mostly neutral salt solutions, upon the blood vessels and the circulation, is due to the action of the kations, but the mechanism of this action is doubtless most complex and difficult to unravel. Harvey (17) warns us that "functional changes cannot be used as a criterion of permeability." It may well be that some of these substances, the strong alkalies and acids, lead, barium, and other metals, destroy the surface properties of the cells, and, as Loeb (23, 25) has suggested, enter into chemical combinations with some components of the protoplasm and thus tend to alter its properties.

Potassium iodide  $\frac{\text{mol}}{20}$  though apparently neutral causes an immediate and rather violent, one might say, spasmodic, contraction which however, is only quite transitory. After a very short while the vessels resume their normal dimensions and functions. Potassium bromide acts similarly, but not quite so violently. Some hydrolysis of the salts had taken place in these solutions, but as the hydrogen-ion concentration after all, was in the neighborhood of  $\text{pH} = 5$ , it does not seem probable that the potassium causes these constrictions and one might think of the possibility of such action on the part of iodine and bromine.

For the purpose of studying these phenomena under somewhat different conditions a series of experiments was carried out according to the method initiated by O. B. Meyer (31) and developed and modified by Voegtlin and Macht (42). For further details of the method, the reader is referred to the latter publication. In our experiments a chain of 4 rings cut from the carotis of a freshly slaughtered ox, and immersed in Ringer's fluid, at a constant temperature between  $37^{\circ}$  and  $38^{\circ}$ , was attached to a lever writing upon a drum which completed one revolution in about 8 hours. The adjustment employed magnified about six and one-third times. The results obtained with alkalies in this series of experiments, coincided very satisfactorily with the observations on the mesentery of the frog. Hydroxyl concentrations of  $\text{pH} = 12$  drove the lever upward almost instantaneously and with such force as frequently to throw it beyond the

upper margin of the drum. Lower concentrations acted proportionately less violently and more gradually. With acids our experience was somewhat different. It was impossible to obtain any definite dilation or relaxation of the tonus with any acid or with any degree of dilution. On the other hand the constricting effect of acid solutions was readily obtained and agreed perfectly with the results on the mesentery. The results obtained with various salts, neutral and otherwise, also agree well with those from the mesentery. Figures 4, 5, and 6 will serve to illustrate these findings.

It is perhaps permissible to conclude from this that alkalies and the stronger concentrations of acids, act in some way directly upon the muscle cells of the blood vessels, and cause them to contract, while the inhibitory effect on the vascular tonus, the power to dilate blood vessels to a certain extent, and accelerate the circulation, which very high dilutions of acids undoubtedly possess, seems to depend upon stimulation of the vaso-dilators. This hypothesis, if true at all, does probably not apply to the entire vascular system. The statement of Alexander Monroe (1783) that vaso-motor nerves are not to be found in the brain, has since been confirmed by some (Roy and Sherrington (36), denied by others, but the researches of Langendorff (22) speak very strongly for the chemical regulation of the blood supply in the brain and mainly by the products of its own metabolism. There is considerable evidence that smooth muscle reacts differently upon stimulation in different organs. The muscles of the stomach, intestines, ureter, uterus, etc., are probably adjusted to specific reactions upon stimulation by their own specific metabolites; by endocrine products; etc., and all of these may well act specifically also upon the special vascular apparatus of each of these organs.

As much has been written, of late, about spontaneous movements of the blood vessels entirely independent of the action of the heart (Gruetzner (12), Hasebroek (18), Homberger (13), Full (9), Severini (37), Mathes (30) and numerous others) and as the subject is still under discussion, it may be well to mention, just in passing and without any intention of entering into the



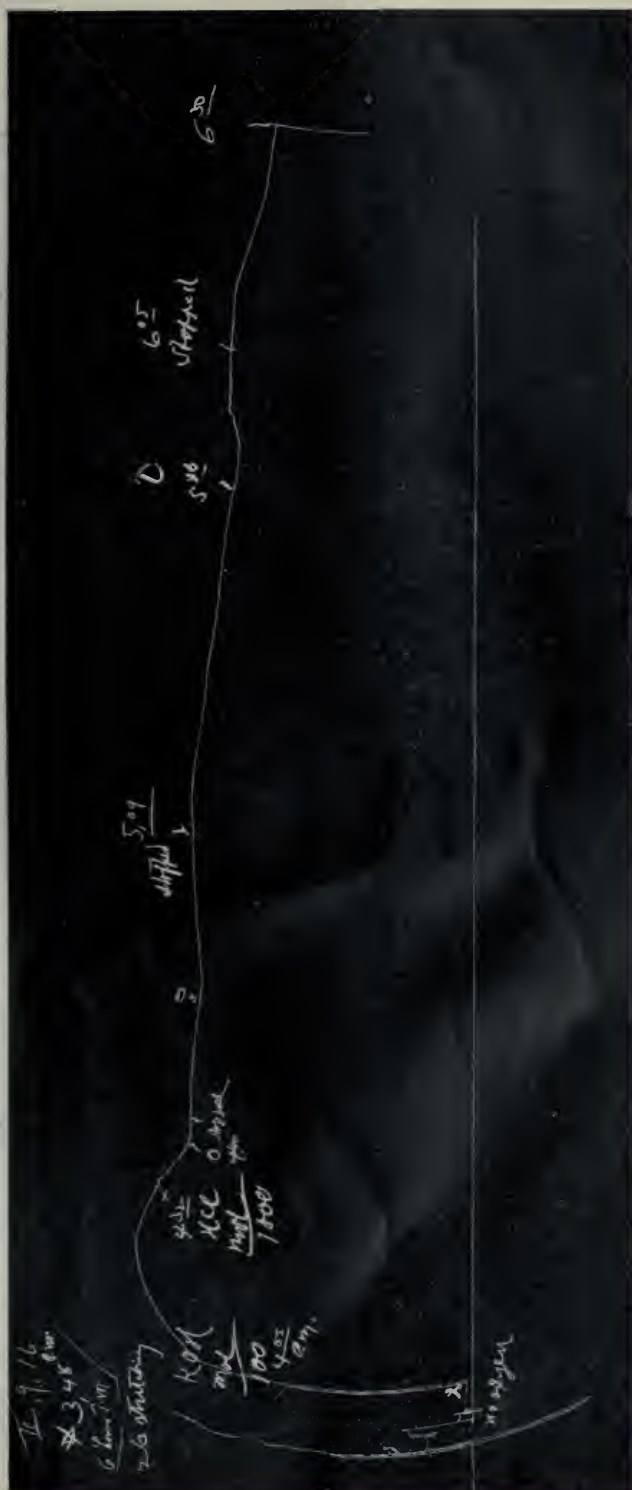


FIG. 4. Shows the prompt and vigorous contraction after KOH in concentration of  $\text{pH}^{12}$  and the absence of any relaxing effect of  $\text{HCl}$   $\frac{\text{mol}}{100}$   $\text{pH}^3$



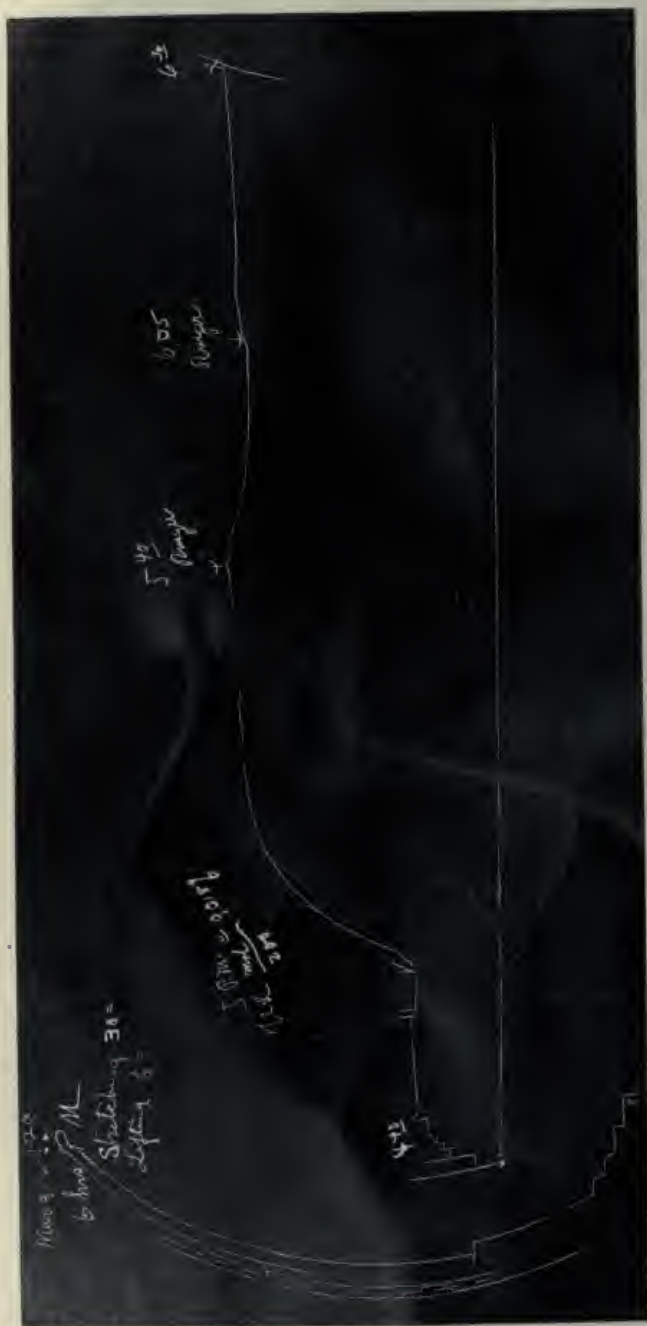


FIG. 5. Shows the gradual constricting effect of  $\text{HCl}$   $\frac{\text{mol}}{200}$



Fig. 6. Shows the prompt and vigorous contracting effect of sodium carbonate and the failure of lactic acid in thin dilutions to produce any relaxation

controversy, that it has been possible on a number of occasions to see the arteries, and in a lesser degree the veins, dilate and contract rhythmically at intervals of a fraction of a minute. The change of volume amounted at the very utmost to not more than 1 space of the micrometer scale. The conditions which might favor these spontaneous movements were not investigated.

As a further illustration of the methods employed some few and characteristic of the very numerous protocols are subjoined.

*Experiment 56.* Frog decerebrated and pithed. Artery measures about 5. Application of sodium hydrate  $\frac{\text{mol}}{20}$ . After about 7 minutes artery measures 3, while the veins which have been much narrowed and have shown considerable "beading" are recovering. After another 3 minutes the artery beginning to dilate, another application of the same solution in the same place. Again after 3 minutes the artery is considerably beaded, measuring at the narrowest places less than 1 and in the interspaces about 3. The adjacent veins also much narrowed. The current in the artery almost completely stopped. Washing with normal saline. After about 6 minutes, veins almost normal, arteries still beaded and about same measurements. Lactic acid  $\frac{\text{mol}}{200}$  pH = 3.08 applied. After 2 minutes the current in the arteries is distinctly more lively and the constrictions very slowly begin to loosen. Within the next 4 minutes, 2 applications of the same solution of lactic acid are made. The point of greatest constriction has dilated from 1 to about 3 and the interspaces to about 4. Some of the capillaries are resuming their current. After 10 minutes artery has slowly almost regained its normal measure and the capillaries are active. The field is carefully dried with litmus paper and a local application of potassium hydrate  $\frac{\text{mol}}{20}$  is made to another artery which shows immediate slowing of the current and narrows down to almost filiform for some distance above and below the point of contact. Circulation in capillaries has stopped. Current in the constricted artery is swift, but the lumen of the vessel which originally was a little more than 5 has narrowed down to about 1 without any "beading." After 7 minutes dilatation is beginning, but considerable "beading" appears. After another 3 minutes the artery is recovering, lumen almost 3.

Washing with lactic acid  $\frac{\text{mol}}{200} = \text{pH } 3.08$ . Artery dilates almost at once, the current in both arteries and veins is very lively, in the capillaries it is slowly being resumed. After 10 minutes, another washing with lactic acid and after 3 minutes more, the potassium artery is fairly normal, the sodium artery still shows some signs of constriction.

*Experiment 68.* Frog is curarized. Artery selected measures between 5 and 6 just above bifurcation. A vein to the left measures 5, to the right one that measures 4. Application of  $\frac{\text{mol}}{20}$  neutral calcium chloride, the current is somewhat slower. After 5 minutes another application of the same solution without any effect. After 6 minutes the mesentery is dried with litmus paper which has no effect upon the current or the measurements of the arteries and shows no reaction.

After 4 minutes application of  $\frac{\text{mol}}{20}$  neutral magnesium sulphate. After another 5 minutes another application of the same solution. After about 17 minutes there is no change. Measurements are the same, blood current even in the capillaries is excellent. The mesentery is dried with litmus paper which shows neutral reaction, and there is no effect upon the current or the vessels. After about further 8 minutes, conditions being perfectly normal, application is made of potassium iodide  $\frac{\text{mol}}{20}$ . Within a minute the blood current racing along with con-

siderable velocity, the artery is violently constricted from the periphery towards the center. At the original point of measure. it is barely 3. The vein is hardly affected. After about a minute, the artery has practically assumed its normal dimensions. After 5 minutes another application of the same solution of potassium iodide was made and immediately again the artery is narrowed from periphery towards the center so that at point of greatest constriction, it measures a little more than 1. The vein again does not seem to be touched. Within a minute the artery begins to dilate again, but the recovery this second time is not as rapid as at first. 10 minutes later everything is normal, the artery having long before regained its normal dimensions. The surface of the mesentery is dried with filtering paper without any effect upon the current or the vessels.  $\frac{\text{Mol}}{20}$  potassium bromide is applied. Imme-

diately thereupon there is a very slight narrowing of the artery and also of the vein. The blood current everywhere is perfect. After



another 5 minutes the vein having now recovered, another application of the same solution of potassium bromide is made. Again there is an immediate narrowing of the vein and also a very slight, hardly measurable, narrowing of the artery. After 10 minutes the artery has recovered completely, but the vein is still considerably narrowed measuring over a great part of its length barely 2, and its current is very sluggish. The mesentery is dried with filtering paper which has no disturbing influence. After 5 minutes the vein is no longer uniformly narrowed, but begins to dilate, showing still considerable constriction. The artery and capillaries are normal, and the blood current everywhere good.

Application is made of formic acid  $\frac{\text{mol}}{100}$  pH = 2.9. After 2 minutes artery is distinctly narrowed, now barely 3, with considerable "beading" throughout its entire extent. The vein is slowly dilating. After another 3 minutes, the vein is still not normal, though dilating, the artery about the same, the current very swift. Another application of the same solution of formic acid. There is no further narrowing of the artery which is much beaded but seems as if it were beginning to recover. The vein is still slowly dilating. All the capillaries are working. After another 5 minutes the vein is almost normal, the artery undoubtedly recovering, having in some places its normal measurement and at places of greatest constriction, now measuring about 4. Blood current in arteries, veins and capillaries, excellent. Experiment which has lasted nearly 2 hours closed.

*Experiment 80.* Frog decerebrated and pithed. Normal measure of artery a little more than 6. Hydrochloric acid  $\frac{\text{mol}}{100}$  pH = 2 applied and within 1 minute, artery is nearly 8. After 4 minutes again normal dimensions. After wiping with blue litmus which fails to show acid reaction and application of normal saline, artery is nearly 7. A second application of  $\frac{\text{mol}}{100}$  hydrochloric acid and within a minute artery nearly 8. Current in the small capillaries seems occasionally to stop until a small lump of agglutinated red cells is thrust through with great force when the current becomes normal again. This process is repeated again and again but gives place to normal conditions after about 5 minutes. The artery now has its normal dimensions. Blue litmus is not reddened. Normal saline is applied and the artery narrows somewhat. After several minutes, application of  $\frac{\text{mol}}{100}$  sodium

hydrate is followed immediately by slight beading of both artery and vein. After 3 minutes artery 5. After another 6 minutes application of  $\frac{\text{mol}}{100}$  hydrochloric acid. Current at once becomes more rapid and artery normal. Beading has disappeared, but after another 2 minutes the artery narrows down to 5. After still another 3 minutes the surface of the mesentery still reddens blue litmus and the artery remains at 5. 6 minutes thereafter  $\frac{\text{mol}}{80}$  sodium hydrate. Artery is reduced to 4 with pronounced beading. After a couple of minutes the artery spontaneously dilates to nearly 5. In the course of the last 10 minutes, it can be observed that without any external interference there is a rhythmical contraction and dilatation of the artery alternating at half minute intervals. After another few minutes application of  $\frac{\text{mol}}{25}$  hydrochloric acid. Current in capillaries stops immediately, but beading disappears, the vessels begin to dilate and after about 2 minutes the artery measures 6, but after another 2 minutes an entirely spontaneously narrowing begins so that after about 7 minutes, the artery measures not quite 4. 15 minutes later washing with normal saline which causes immediate but temporary stoppage of all current and a narrowing of the artery to a little over 3. About 10 minutes thereafter the current in arteries and veins has regained normal velocity but in the capillaries, it has not yet recovered. The measurement of the artery is now a little more than 5 and remains so. Experiment has lasted about 2 hours.

*Experiment 71.* Frog decerebrated and pithed. Brilliant current in arteries, veins and capillaries. In order to test the effect of mere mechanical irritation, a thread that had never been used, but was simply moistened with normal saline is employed to stroke and push about some of the larger arteries and adjoining veins for a greater part of a minute. No effect was visible on the artery, but some of the veins responded by some narrowing and constriction, but no beading. This purely mechanical effect passes very promptly and in a very short time the vein resumes its normal dimensions without any alteration in the current. As soon as everything had become entirely normal, a local application is made to one of the larger arteries of  $\frac{\text{mol}}{20}$  barium nitrate. The current at once both in the artery and adjoining veins becomes slow and oscillates. There is no current in the surrounding capillaries.

In about 2 minutes a number of veins collapse and become almost entirely empty. It takes fully 5 minutes however before the artery shows any effect, then there is almost complete closure and the current is extremely slow. After about 5 minutes washing with normal saline, but the constriction goes on and the artery now, about 10 minutes after the application of the barium, is simply a straight line with a thin thread of red corpuscles in single file passing through it. There are constrictions of various degrees in the veins. 15 minutes thereafter the effect of the barium is still unaltered, the current extremely sluggish and more or less contraction of all vessels. The surface of the mesentery is dried with filtering paper and again normal saline applied. After another 15 minutes the original artery is almost normal, the veins less so, but much improved. The current is very slow and more or less irregular. Application is made of  $\frac{\text{mol}}{1000}$  lactic acid  $\text{pH} = 3.4$ . In about minute the current is decidedly stimulated, the constricted veins are beginning to dilate and some of the capillaries which have been empty are showing current again. After another 5 minutes another application of  $\frac{\text{mol}}{1000}$  lactic acid is made. The current remains good and the surface is dried after a while with filter paper and washed with normal saline. After about 15 minutes, the current everywhere is somewhat slow, but fairly regular. The mesentery is again dried with filter paper and a local application is made of a 2 per cent solution of NaCl. Almost instantaneously the current in the capillaries, arteries and veins, large and small, stops completely without any constriction, so that all the vessels are gorged with blood and the mesentery looks as if it had been artificially injected. After 5 minutes, the mesentery is dried with filter paper and then washed with normal saline. After about 10 minutes the circulation appears to be starting up again in some of the arteries and veins and in many of the capillaries, and it seems that if time were given, the circulation would recover.

Summing up results the writer is fully conscious that there still remain sufficient problems that have not been solved and discrepancies that have not been reconciled. The curiously uneven and apparently irregular contraction of the vascular walls which we have called "beading," demands further investigation and explanation. The fact that cleansing the mesentery with filter or litmus paper in one instance causes no disturbance what-



soever, and that again violent constrictions may follow the same manipulation, though suggesting several plausible hypotheses, has not attained a precise explanation. Similarly it has often been observed that acid solutions (alkaline not so frequently) had apparently no effect until the mesentery was washed with normal saline, when the constrictions promptly appeared. Here again several explanations suggest themselves, but all these problems await further investigations. Nevertheless it is believed that certain conclusions may safely be drawn from our observations. They furnish a further corroboration, of what may now be accepted as a fact, that "there is, in the words of Bayliss, a complete chemical regulation of the cardio-vascular system which may act independently from the central nervous system." It has here been shown also that it is not only the blood circulating in the blood vessels, that takes part in the regulation of their functions, but that the chemical conditions of the tissues and fluids surrounding the blood vessels also exercise an important influence. It appears furthermore that it is not only the products of the internal secretions which are concerned in this, but that common acids and alkalies; the specific metabolites of the tissues and cells; besides a number of neutral salts, under ordinary circumstances foreign to the organism, may exercise a controlling influence. Strong acids act like alkali in compelling vessels to contract. It is true, that such extreme chemical changes as have here been presented are ordinarily not likely to occur. Rowntree (27) and his associates estimate the hydrogen-ion concentration of normal human blood plasma as varying from  $\text{pH} = 7.4-7.6$ ; of the blood serum from  $\text{pH} = 7.6-7.8$ . In clinical acidosis  $\text{pH} = 7.3-7.1$  and in dogs just before death from experimental acidosis  $\text{pH} = 6.9$ . Henderson (21) has shown that the blood is able to dispose of considerable quantities of alkali or acid without much change in its reaction. There must necessarily however be a limit to this, and one may conclude from the observations here recorded that besides the chemical and physical reactions of the blood itself, there are other chemical and physical factors which, when once a certain limit has been overstepped, may lead to grave disturbances of circu-



lation, and one can easily conceive of certain pathological processes such as inflammations, thromboses, necroses and other local, possibly even systemic, affections originating in this manner.

I wish to express my thanks to Dr. K. G. Falk of the Harri-man Research Laboratories to whom I am indebted for valuable help in the providing of accurate solutions and in the determination of hydrogen-ion concentrations.

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## ON THE ACTION OF ATROPINE SULPHATE ON THE ISOLATED STOMACH AND BOWEL OF THE DOG

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The effects of atropine on the small intestine have been the subject of some discussion. Magnus (1) ascribes to this alkaloid a double action, both paralysing and exciting the movements of the stomach and intestine. The excitor action he believes to be due to action upon the nervous plexuses, for it does not occur when Auerbach's plexus is removed; after this large doses of atropine paralyse the movements. Otto Herz (2) has shown that the exciting action of small doses of atropine is rarely present in the isolated intestine of the cat, and never in the isolated intestine of the rabbit. Now Henriquez and Hallion (3) found a substance in the small intestine of the dog which excites peristalsis, and Weiland (4) has improved their methods of analysing the action of this substance which he calls "motiline." One of us has confirmed, along with György (5), the technique of Weiland, and has applied it to the study of the action of morphine on the small intestine. We thought that there would be some interest in examining the action of atropine on the movements of the isolated bowel of the dog and on the production of this substance. In addition, we have extended the investigation to the action of atropine on the isolated stomach.

Owing to circumstances beyond our control, we have been obliged to interrupt these studies before their completion, and as some time may elapse before we can recommence them and as one of us (6) has already published two communications on the subject, we have thought it advisable to put on record the principal results which we obtained during the academic year 1913-1914.

*Methods.* The dogs received a hypodermic injection of a watery solution of atropine sulphate amounting to 1 mg., 0.1 mg.,



0.005 mg., or 0.001 mg. per kilo in different experiments. Except in the first experiments we used two dogs in each, the dogs having fasted since the evening before the experiment. The one served as a control, the other received atropine from half an hour to nine hours before the experiment. The abdomen was opened under anaesthesia with equal parts of alcohol, ether and chloroform. A portion of the small intestine was removed and carefully washed in Tyrode's solution at a temperature of 32°C. and then a loop 3 cm. in length was ligatured at both ends and put in Neukirch's apparatus (7) with two lateral tubes, so that the whole was covered by the solution of Tyrode at 38°C., and the upper end only reached half way through the fluid. The receiver contained altogether 100 cc. of liquid, and oxygen was bubbled through it throughout the experiment. The whole apparatus was placed in a thermostat at 38°C. The lower end of the gut was attached to a glass hook at the bottom of the vessel, while the upper end was attached to an aluminum writing lever. In each experiment two of these loops were prepared, one from the normal dog and one from the atropinised dog.

Further, two beakers were placed in a thermostat at 32°C. each containing 50 to 100 cc. of Tyrode's fluid. In the one was placed an intestinal loop from the normal dog, 15 to 20 cm. in length ligatured at both ends, and in the other a similar loop from the atropinised dog. After half an hour to an hour the loops were withdrawn from the beakers which were heated to 38°C. in a thermostat. These we shall term extract of normal intestine and extract of atropinised intestine respectively.

In a considerable number of experiments we have removed the stomach, washed it carefully with Tyrode's fluid, then ligatured it at each end and attached one end to a writing lever in the same way as for the intestine. In some cases we prepared an extract of normal stomach in the same way as that previously described for the intestine.

In some experiments we did not use a control dog and merely examined the changes in the atropinised ones.

In the majority of our experiments we commenced by registering the movements of the two loops for some time and then

examined the effects of the action of the atropinised extract on the normal loop and on the atropinised loop. We also examined in some cases the action of these extracts on the stomach of a normal and of an atropinised dog.

The substitution of the extract of intestine for the ordinary Tyrode's fluid was effected as follows: a syringe with a capacity of 100 cc. was fitted to the second lateral tube of Neukirch's apparatus. The liquid was removed very slowly by means of the syringe until it reached only a few millimeters above the upper end of the loop. The extract to be examined was then added in an amount equal to that withdrawn by the syringe. This was repeated three times, care being taken not to expose the loop for a moment to the air.

Further we may mention that we have studied in some experiments the effects of the addition of 1 mg. of atropine sulphate to the fluid in which a normal loop was immersed.

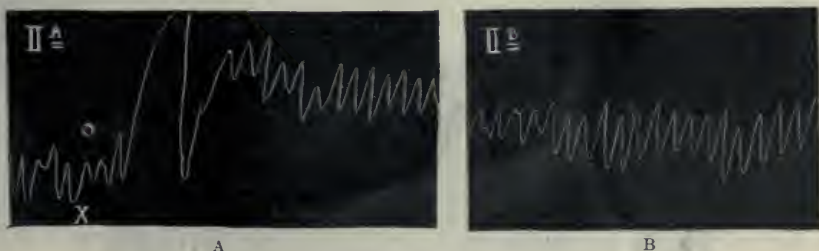


FIG. 1. NORMAL INTESTINAL LOOP

At *x*, extract of normal intestine was substituted for Tyrode's fluid. Between A and B there was an interval of one hour.

#### EXPERIMENTS ON THE INTESTINAL LOOPS OF NORMAL DOGS

We have confirmed the statement of Weiland that the addition of the normal intestine extract, motiline, to the Tyrode's solution increases the amplitude of the contractions of an intestinal loop. There is also a slight increase in tonus. This augmentor effect on the tone and on the amplitude of the contractions persists for an hour or even sometimes for three hours (fig. 1, A and B).

The normal stomach extract as a general rule has no effect on the contractions of an isolated intestinal loop. In one case how-

ever we saw a rapid fall of tone and a slight diminution of the contractions.

The atropinised extract has the same effect as the normal extract on the amplitude of the contractions of the normal gut. In both cases the amplitude is markedly increased. On the other hand, the atropinised extract has no effect upon the tonus or the rhythm of the normal intestinal loop. This is true whether the animal had received 1 mg. per kilo of atropine or smaller doses down to 0.001 mg. (figs. 2 and 3).



FIG. 2. NORMAL INTESTINAL LOOP

At x, extract of atropinised intestine.



FIG. 3. NORMAL LOOP

At x, extract of atropinised intestine.

#### EXPERIMENTS ON THE INTESTINAL LOOPS OF ATROPINISED DOGS

Isolated loops of the intestines of dogs which had received half an hour before a hypodermic injection of 1 mg. of atropine only show very small movements and lose their tone rapidly. While the normal intestinal loop contracts for several hours, the atropinised loop shows no contraction except for a few minutes. But the



addition of atropinised extract causes a strong and rapid augmentation of tone, which however does not last for long. The amplitude of the contractions tends to increase gradually.

Four hours after the injection of 1 mg. of atropine the intestinal loop hardly contracts at all. The addition of atropinised extract increases the amplitude of the contractions, and later causes a rise in tone (fig. 4).



FIG. 4. INTESTINAL LOOP

From dog injected four hours previously with one mg. atropine sulphate per kg. At x. extract of atropinised intestine.

The intestine isolated five hours after the hypodermic injection of 1 mg. of atropine does not present numerous or very strong contractions and the tone falls rapidly. Atropinised extract remains without effect for some time and does not arrest the fall of tonus or the diminution in the movement. Finally the size of the contraction increases very slowly and gradually. After a considerable time the tone begins to rise and the contractions increase in size.

Five and a half hours after the atropine injection the intestine presents the same contractions as the normal, and the tone main-



tains its initial height for a relatively long time. Atropinised extract now causes a rapid rise in tone followed by a rapid fall, and afterwards a new rise. The size of the contractions is very irregular both before and after the administration of the atropinised extract (fig. 5).

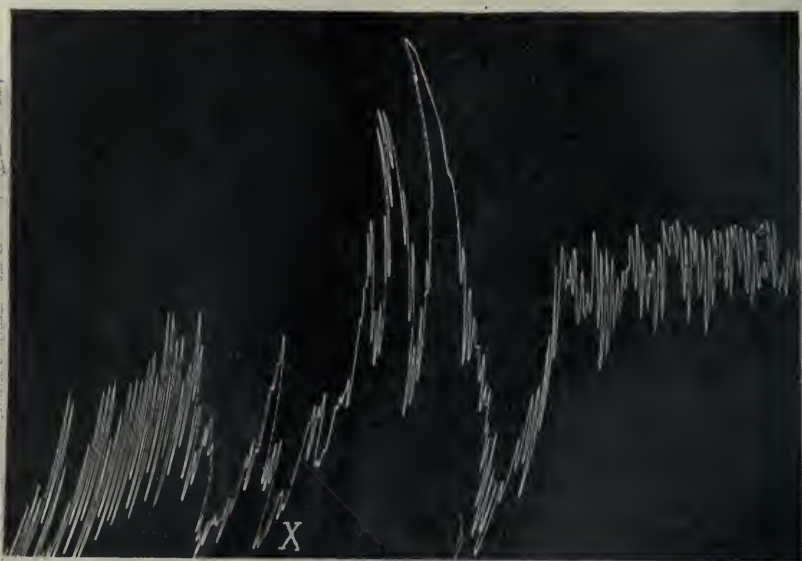


FIG. 5. INTESTINAL LOOP

From a dog injected  $5\frac{1}{2}$  hours previously with 1 mg. atropine per kg. At x, extract of atropinised intestine.

As a general rule seven hours after the administration of atropine the intestine contracts and reacts to the intestinal extract in the same way as the normal loop, but some animals seem to remain longer under the influence of the alkaloid. For instance, in one case the bowel hardly contracted seven hours after the atropine; and the extract remained without effect for eighteen minutes. In another animal the intestine, isolated nine hours after the injection of atropine, presented a fall in tone but an increase in the contractions after the administration of the extract (fig. 6).

In dogs atropinised with 0.1 mg. per kilo the effects on the intestine resembled strongly those caused by 1 mg. per kilo. The

changes are perhaps less marked after the smaller dose. Under the influence of intestinal extract, the size of the contractions becomes gradually larger and the rhythm becomes more irregular. 0.005 mg. per kilo still causes a considerable diminution of the motor activity and a marked fall in the tone of the intestinal loop isolated half an hour after the subcutaneous injection.

The application of the atropinised extract causes an increase in tone and in amplitude, but just as in loops subjected to larger doses of the alkaloid, the tone returns relatively quickly to the level maintained before the extract was applied. The size of the contractions sometimes even diminishes during this fall in tone,



FIG. 6. INTESTINAL LOOP

From a dog injected 9 hours previously with 1 mg. atropine per kg. At *x*, extract of atropinised intestine.

but this is only a transient phase for after a few minutes the amplitude of the contractions increases markedly. Then the tone begins to rise and remains at the high level at which it had previously arrived. The intestinal loops of those dogs subjected to 0.005 mg. atropine per kilo show more distinctly and more intensely the effects of the motiline than loops removed from animals previously treated with larger doses of atropine.

0.001 mg. atropine per kilo has no effect upon the contractions, but as in animals subjected to larger doses, the tone always falls



FIG. 7. INTESTINAL LOOP

From a dog injected  $\frac{1}{2}$  hour previously with 0.005 mg. atropine per kg. At *x*, extract of atropinised intestine.

rapidly (fig. 7). The atropinised extract causes a rapid increase in tone which returns rather rapidly to the previous height. Then after a short pause at this height, it soon begins a further and marked rise which is permanent. .

The normal extract of intestine has exactly the same effect as the atropinised extract when it is applied to loops from an animal which has received the atropine half an hour, or even four hours previously. But in loops from an animal treated five to five and a half hours or longer previously with 1 mg. of atropine the addi-

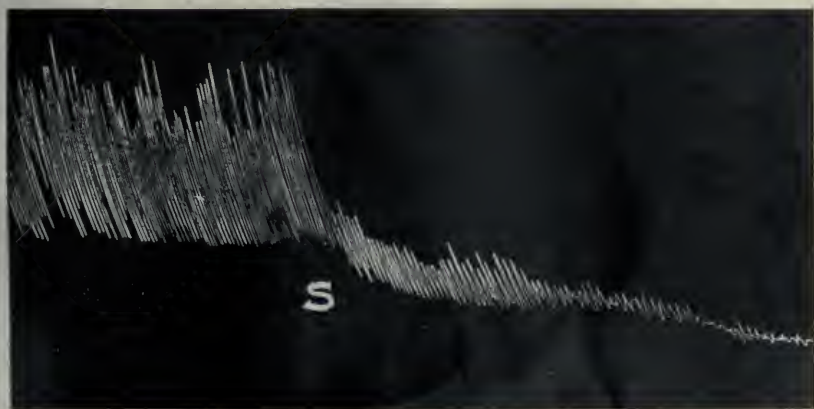


FIG. 8. NORMAL LOOP

At S, 1 mg. of atropine was added to the Tyrode's fluid.

tion of normal extract causes a gradual and regular increase in tone and in size of the contractions without the momentary fall of tone which is intercalated between two elevations under atropinised extract.

Thus the intestine of an atropinised dog reacts normally after a shorter time under normal extract than under atropinised extract. The addition of 1 mg. atropine to 100 cc. of the Tyrode's fluid containing a normal intestinal loop causes an immediate fall in the size of the contractions and also in tone (fig. 8).



## ACTION ON THE STOMACH

The dog's stomach treated as described above shows much fewer contractions than the intestine, and these diminish in size after some time and cease altogether in from fifteen minutes to one hour or longer. The tone tends to fall slowly as the amplitude decreases. The addition of normal extract of stomach or intestine does not seem to exercise any action on the contractions.

The stomachs of dogs which have received 1 mg. atropine per kilo half an hour previously, present very few and very feeble contractions which lessen gradually. The tone falls slowly. Four hours after the hypodermic injection of 1 mg. of atropine, the isolated stomach loses its tone rapidly and the size of the contractions falls rapidly. Six hours after the injection the contractions are more distinct, and the organ maintains its tone. Still later—six and one-half to nine hours after the injection—the contractions are as large as normal but present some inequalities. The tone only falls slowly as in the normal condition. The atropinised or normal intestinal extract has no distinct effect. In one case however, the atropinised intestinal extract immediately caused a slight fall in tone which hardly increased afterwards.

## DISCUSSION

The intestinal loops of a dog which has previously received atropine only contract feebly, do not retain their tone, and rapidly cease moving. This may be observed half an hour after the injection of 1 mg. and appears to persist as a general rule for five and one-half hours or sometimes seven hours or even longer. The action of doses of 0.1 and 0.005 mg. per kilo is similar and our results do not suffice to establish whether this action is more transient with these smaller doses, though it seems probable. Half an hour after 0.001 mg. per kilo there is no effect observable on the movement, but the rapid fall in tone is still noticeable.

Atropine by hypodermic injection therefore seems to act more strongly on the tone than on the contractions.

It may be recalled that the time during which food remains in

the stomach and in the small intestine is not affected by 0.001 mg. atropine per kilo in the dog, while 0.005 mg. and larger amounts cause the food to remain longer in the stomach.

Thus when there is no interference with the movement, the period during which the food remains in the gastro-intestinal canal remains normal. The changes in tone hardly seem to influence the rate of the digestive processes.

It appears to follow from our experiments that the gut forms both in atropinised and in untreated animals a substance possessing the property of augmenting the size of the contractions of the intestine isolated from a normal dog. But the atropinised extract has a less noticeable action in increasing the tone than the normal extract. This is undoubtedly due to the presence of a small amount of atropine in the extract obtained from the atropinised animal.

Normal or atropinised extract has a distinct effect upon the isolated gut of the atropinised dog, so that the substance exciting peristalsis continues to act in these animals, but the action is more irregular and appears only after a shorter or longer time and in successive steps. This difference in the action of the atropinised extract from that of the normal is probably due to the presence of atropine persisting in the Tyrode's fluid, for the action of atropine on the tone and in a smaller degree on the movements of the intestine antagonises the action of the motiline. But this action of atropine is temporary, and the motiline soon regains its efficacy and the tone and the size of the contractions increase again.

When the extract is prepared from a loop removed half an hour after the injection of 1 mg. atropine, one sometimes observes depressant effects of the atropine on the movement and the tone a second time.

These considerations must be however regarded as purely hypothetical for we have not been able to demonstrate quite certainly either chemically or physiologically the presence of atropine when the atropinised extract was concentrated down, but unfortunately we have not followed this line of research systematically. It would be necessary first to observe if the salts

contained in the Tyrode's fluid tend to lessen the sensitiveness of these reactions to some extent.

We have already remarked that the addition of 1 mg. of atropine to the bath lessens rapidly the size of the contractions and causes a fall in tone of the intestine. It would be of interest to determine the smallest dose of the alkaloid which possesses these depressant effects, as one could thus determine definitely if the action of the atropine really is stronger upon the tone than upon the movement of the intestine, as our experiments seem to show. If this is the case and if the action on the tonus is exercised by extremely feeble doses, it would perhaps be of interest to employ intestinal loops to determine physiologically the presence of very small quantities of atropine. It would be premature however to make a statement at present on this subject, which requires further examination.

The action of atropine on the stomach does not call for special remarks.

One milligram seems to exercise the same depressant action on the movement and on the tone of the stomach as of the small intestine.

Contrary however to what we have noted in the intestine, there does not seem any reason to suppose that the atropine acts more strongly on the movement of the stomach, but perhaps this is based upon an insufficient number of experiments.

It is certainly necessary to study the action of atropine on the movement and tone of the gastro-intestinal canal further and to extend the study to other species. The action differs fundamentally from that of morphine, for the latter in doses of 4 to 6 mg. lessens or entirely prevents the formation of the substance which excites peristalsis. Under morphine the intestine reacts at first less and finally not at all to this substance. This failure to react persists for some time until the intestine forms more motiline, when the disturbance of tone and motion disappears.

## SUMMARY

1. The stomach and intestine of the dog isolated half an hour after a hypodermic injection of 1 mg. atropine per kilo only contract weakly and with diminishing strength. The tone falls rapidly. The same is true for the stomach. These effects persist as a general rule for five or six hours or longer, at any rate in the intestine.

2. The same depressant action may be obtained after 0.1 mg. or 0.005 mg., while after 0.001 mg. the movement is normal though the tone falls rapidly.

3. The production of the substance which excites movement and the reaction of the intestine to this substance did not seem to be influenced by the previous injection of atropine.

4. The extract of the stomach or of the intestine has no influence on the contraction or tonus of the isolated stomach.

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# ON THE INCREASE OF "TONE" ASSOCIATED WITH THE ACTION OF STROPHANTHUS ON THE HEART

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The following observations were incidentally made in the course of some experiments designed to test the excitability of the frog (*Rana temporaria*) ventricle when under the influence of strophanthus. The apparatus used was the same as that employed by Tait (1) in experiments on the action of yohimbine on the heart. A Schäfer frog-heart plethysmograph with Ringer's solution in its lower half and fluid paraffin in its upper half (including the horizontal arms) recorded the movement. The apex of the ventricle dipping into the Ringer's solution, a break induction current could be sent at any given moment from this electrolyte through the heart-wall to the paraffin-surrounded metal cannula to which the organ was tied. The point of stimulation was signalled on the tracing by a short succession of sparks from a sparking-coil.

In an advanced stage of strophanthus action, as is well known, the beats of the ventricle become increasingly slowed, while at the same time the "tone" of the muscle progressively rises. The result is that each successive beat, being superposed upon a gradually heightening tone, is of less total extent than its predecessor. One of the original objects of inquiry was to discover if the heart is refractory during the stage of tonus contraction. It was found that the rapid contraction superposed upon the slower preliminary (or tonus) contraction is alone an active contraction of the muscle accompanied by a refractory phase. The tonus tightening of the ventricle involves no refractoriness (see fig. 1.) These observations confirm the gen-

eral opinion, first expressed by Schmiedeberg (2), that the two forms of contraction are of quite different nature.

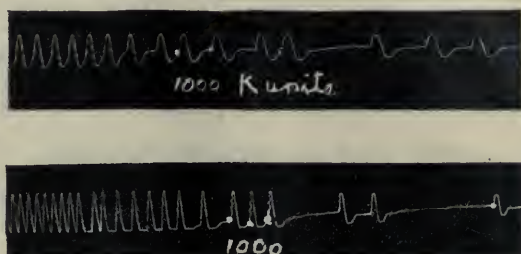


FIG. 1. To show absence of refractory state during the stage of slow (or tonus) contraction of the strophanthinised ventricle. *All figures to be read from left to right. Systole is recorded in the upward direction.* In both tracings can be seen the rapid development of a slow tonus contraction, upon which the ordinary beats are superposed. Electrical stimulation at any phase of the tonus contraction is effective in causing a beat of the ventricle. In every case the strophanthus was dissolved in Kronecker's ox-blood mixture for the frog-heart; concentration in experiment of upper tracing—1 in 100,000, in experiment of lower tracing—1 in 200,000. Stimulation with break induced shock from a Kronecker coil, two volts in primary circuit. Figure reduced by one-half.



FIG. 2. To show a peculiar irregularity in the beat of the deeply strophanthinised ventricle. Rate of beat, amplitude of contraction, amplitude of relaxation, are all irregular. Between the extent of contraction however and that of the succeeding relaxation there is in each case a marked correspondence; the amount of the latter varies directly with that of the former. The upper tracing shows points at which electrical stimuli were applied; this however has nothing to do with the essential phenomenon. Concentration of strophanthus, 1 in 400,000. Perfusion-pressure, 9 inches, i.e., 23.5 cm. Figure reduced by one-half.

During this stage the ventricle may beat irregularly. In figure 2 are shown two examples from one and the same ventricle. In the first of these, pronounced irregularity followed upon elec-

trical stimulation of the heart; in the second, the irregularity developed spontaneously. In both tracings the rate of beat is irregular, the amplitude of contraction varies, and the amount of diastolic expansion also varies. Inspection of either tracing reveals one invariable feature however, viz., that the extent of diastolic expansion varies directly with the height of the preceding systolic contraction. It is to this regular feature in the irregularity that we would particularly invite attention.

To account for it, one might conceivably frame the hypothesis that maintenance of "tone" demands a continuous supply of energy; if a given heart-beat makes a large demand on the total supply of energy available at the moment, tone correspondingly suffers. On the other hand, to avoid the assumption that energy is consumed in maintaining tone, one might seek an explanation of the phenomenon in variations of the pressure conditions within the (elastic) heart. It is not at first sight a simple matter to frame a satisfactory hypothesis along such lines, because the perfusion-pressure in the apparatus remained constant throughout.

We decided to vary the pressure. When it was removed from the interior of the heart-chamber (this is readily achieved by clipping the ingoing perfusion-tube) relaxation of the muscle did not occur after contraction. This fact, which applies not only to the strophanthus-perfused but also to the Ringer- or blood-perfused ventricle, is of course well known. It was fully discussed by Roy (3) in the communication in which he first described his frog-heart tonometer. In the same communication Roy confirmed the fact, already pointed out by Schmiedeberg, that the degree of distension of the resting digitalis-ventricle is a function of the pressure applied internally and of the degree of poisoning with the drug.

When the wall of the chamber was now suddenly re-subjected to tension (the ingoing perfusion-tube was released), the ventricle at once became greatly distended, much beyond the mean distension corresponding to the head of pressure and the particular stage of poisoning reached. It then, more slowly, swung back to the position of equilibrium. This result, which is invariable,



does not tally with the first-mentioned hypothesis above; it is impossible to believe that an active contraction, involving temporary diminution of tone, had each time been in progress just before the inlet-tube was unclipped.

Figure 3 shows some of these results. When the inlet-tube is first clipped the plethysmograph piston does not immediately

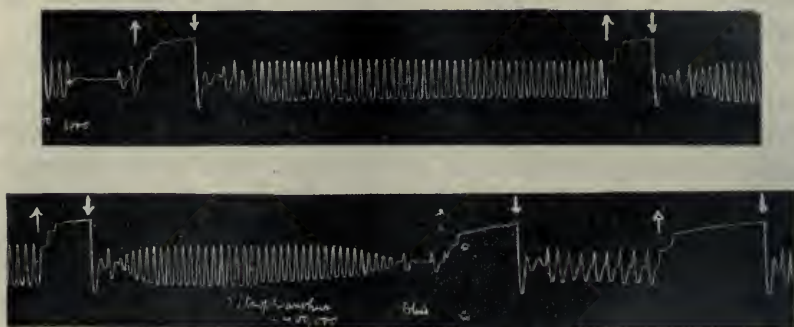


FIG. 3. To show the effect of clamping and then suddenly releasing the inlet perfusion-tube of the strophanthinised ventricle. (The lower tracing is a direct continuation of the upper one. Perfusion-pressure and concentration of strophanthus same as recorded in figure 2.) On five separate occasions the inlet-tube was clamped (at the summit of a systole) and after a little suddenly released. Removal of the perfusion-pressure by clamping allows of a better emptying of the ventricle, which goes on shrinking in size without relaxing. The noteworthy point is that sudden re-admission of the internal pressure causes a great relaxation, greater than when the ventricle is beating without extraneous interference. Until the inlet-tube was just to be clamped for the fourth time the ventricle was perfused with strophanthus; thereafter with blood-mixture alone. Figure reduced by one-half.

attain its full height;<sup>1</sup> at first it rises in successively diminishing steps, which correspond to active contractions; the steps disappear to be replaced by a slow continuous contraction, which, if allowed to proceed, would end presumably in complete occlusion of the ventricular cavity. The absence of steps on this latter part of the trace is due to the fact, pointed out by Schmiedeberg, that a certain minimal internal pressure is necessary to keep

<sup>1</sup> For convenience the word "height" is used in reference to the extent of a horizontal excursion, systole being supposed to drive the piston upwards.

the digitalis (and therefore also the strophanthus) heart beating. Whenever pressure is re-applied to the interior of the chamber, a great expansion is recorded, which exceeds that attained in the natural beating of the poisoned heart. It can also be seen, less clearly in this figure, more distinctly in the succeeding one, that the longer the inlet-tube remains clipped and consequently the more completely the heart is emptied, the more pronounced is the succeeding relaxation on re-application of the internal pressure.

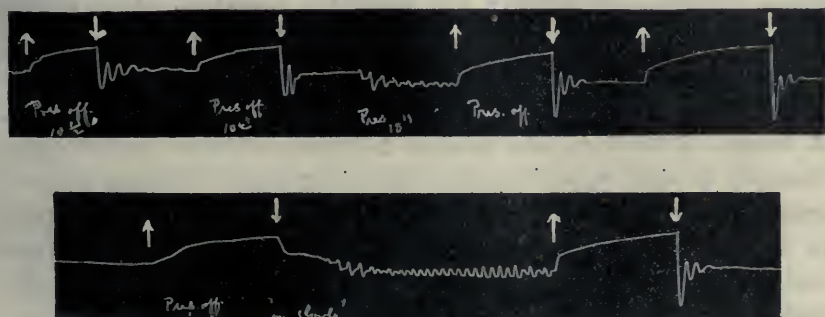


FIG. 4. Showing (1) the experiment of figure 3 at different pressures; (2) the effect of slow decrease and slow increase of the perfusion-pressure; (3) physical oscillations of the strophanthinized ventricle. (The lower tracing is a direct continuation of the upper.) Six separate removals of pressure are recorded. At the time of the first two the head of pressure was  $10\frac{1}{2}$  inches (27 cm.), thereafter 18 inches (45 cm.). On the first four and on the last occasion the inlet-tube was suddenly clipped and suddenly released; on the fifth occasion the perfusion-pressure was slowly removed and slowly restored (bottle moved by hand). Note (1) that the relaxation of the ventricle on sudden re-admission of the higher is greater than that on sudden re-admission of the lower pressure; (2) that with slow re-admission of pressure extreme distension of the ventricle is absent; (3) after-oscillations when the ventricle is suddenly distended (these are physical). Figure reduced by one-half.

In figure 4 two additional variations in the experimental procedure are illustrated. The first of these consists in a repetition of the preceding experiments, only with different heads of pressure. The result is what one might expect; the amount of distension associated with the application of high is greater than that associated with the application of a lower pressure. The second variation is more important for us. Here the pressure acting

on the interior of the chamber was removed slowly, and slowly re-applied, by lowering and raising respectively the bottle containing the perfusion-fluid. With slow re-application of the pressure the extreme distension which we have learned to associate with immediate unclipping of the inlet-tube is absent; the ventricle merely takes up the mean position corresponding to the particular degree of poisoning at the time, and to the fixed pressure eventually attained.

We see therefore that the degree of distension produced by a given perfusion-pressure in the case of a ventricle subjected for a given time to a definite concentration of strophanthus, is not a function of the pressure alone, but also of the *rate of application* of the pressure. This shows that the "tone" is elasticity. A weight applied to the end of a ribbon of steel horizontally clamped at the opposite end, will temporarily deform it to a greater or to a less extent according as it is appended suddenly or more slowly applied. Sudden application of the weight too will set up oscillations which gradually die out. In the ventricle, under favorable conditions, after-waves are visible (see fig. 4); and experiment has satisfied us that at any phase of such after-waves the heart muscle is, as a rule, excitable, in other words that in most cases they are physical oscillations, not muscle contractions.

We shall now consider how the extent of an active contraction of the musculature affects the amount of downward swing of the recording-pen, when the head of pressure acting on the interior of the cavity is constant. Let us refer once more to the case of the steel ribbon clamped at one end, and let us suppose that it has taken up a position of equilibrium on application of a definite weight. If the weight is lifted ever so little and then suddenly allowed to bear on the spring, the weighted end will drop slightly below the point of equilibrium. If the weight is lifted through a greater distance before being suddenly re-applied, the end of the spring will execute a greater excursion.

In the case of the heart the perfusion-pressure corresponds to the weight, the stretched strophanthinised ventricle to the bent spring; any active contraction of the musculature, bringing into



play a new force, which acts against the perfusion-pressure, is analogous to lifting of the weight. Consequently, between the extent of diastolic excursion of the ventricle and the amplitude of the preceding active contraction there will be an unvarying correspondence. For convenience, but not implying the strict mathematical relationship, we have already in our first mention of the phenomenon expressed this by saying that the extent of the former "varies directly with" the extent of the latter.

Lastly, we would refer to another phenomenon connected with elasticity, which is present in the case of the strophanthinised ventricle. While some (elastic) physical bodies recover their form immediately after removal of the distorting force, others, although they recover it ultimately, take a longer time to do so. This delay in recovering the original condition of the substance is known as "elastic after-effect." Reference to figure 1 will show that when, with perfusion of strophanthus, the increase of elasticity first becomes manifest, recovery of the equilibrium position after extension is not immediate. This phenomenon is of course well known to pharmacologists. The influence of elastic after-effect is frequently visible too in the oscillations which follow extreme and rapid variations of the perfusion-pressure, the first rebound of the ventricle being of less height than some of the succeeding oscillations. We could provide tracings in which this is seen on every occasion. The fourth series of oscillations shown in figure 4 may however serve as an illustration.

#### GENERAL CONCLUSION

The peculiar slow contraction, sometimes vaguely referred to as "heightened tone," which in the deeply strophanthinised heart precedes the apparent active contractions, being unassociated with any refractory state, does not involve physiological activity in the muscle. As Schmiedeberg tentatively suggested, the heightened tone is really an increase of elasticity (it will be remembered that the measure of elasticity is the quotient,  $\frac{\text{stress}}{\text{strain}}$ ). Roy, dealing specially with the question of elasticity of the heart,



could not identify the change produced by digitalis (which he classified under "Ideo-muscular Contraction") with an increase of elasticity. With Roy's view we cannot agree. Certain peculiarities in the record of a perfused strophanthus-ventricle find their only explanation in the increase of elasticity produced by the drug.

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# PHARMACOLOGICAL AND CHEMICAL STUDIES ON "SENSO," THE DRIED VENOM OF THE CHINESE TOAD

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## INTRODUCTION

"Senso" is a dried preparation of the skin secretion of the Chinese toad, which is highly esteemed in China as a sovereign cardiotonic and roborant. But very little is known as to the method of its preparation and in regard to the species of toads from which "Senso" is made by the Chinese. As already noted by Prof. H. Hayashi (1) it is similar in its action to digitalis, but is fifty to one hundred times more powerful. Besides this author no one has hitherto attempted to study this product scientifically.

With regard to the secretion of the toad's skin, many investigations have been undertaken from the pharmacological, as well as the chemical point of view. According to Faust (2) the chief principle of the venom of the common European toad is an amorphous substance of acidulous character, readily soluble in aqueous solutions of an alkali. He named it bufotaline and assigned to it the elementary formula,  $C_{34}H_{46}O_{10}$ . This substance is a heart poison with the pharmacological activity of the digitalis group, as was shown by Faust in a series of exact pharmacological investigations. On the other hand, he has described a second substance which he supposed to be present in toad skin which he named bufonine,  $C_{34}H_{54}O_2$ , and which he described as being a chemically neutral and crystalline substance. This is stated to be much less active than bufotaline, but still has a digitalis-like action on the heart.

Phisalix and Bertrand (3) declare that the venom of the European toad contains but one poison of the digitalis type. Their substance, a non-crystalline resin, named bufotaline also, is not of acidulous, but of neutral character. To their bufotaline they ascribed the formula  $C_{119}H_{117}O_{28}$ .

John J. Abel and Macht (4) have isolated in pure form the principles of the skin secretion of *Bufo aqua*. According to these

investigators, the chief principle with the digitalis-like action, is a chemically neutral, crystalline substance which has been named bufagin and to which the elementary formula,  $C_{13}H_{24}O_4$ , has been ascribed. This product was studied by them pharmacologically as well as chemically. They are the first to have isolated the chief principle of the venom of the toad in crystalline form, as far as we know. Besides bufagin, they have proved the existence of epinephrine (adrenaline) in the venom of *Bufo aqua*.

According to Wieland and Weil (5), when Faust's bufotaline is entirely free from adherent suberic acid it no longer possesses an acidulous character and can then readily be purified by repeated crystallization. These investigators have shown that bufotaline, as purified by them, is a chemically neutral crystalline substance whose elementary composition is represented by the formula,  $C_{16}H_{24}O_4$ , and not by the formula,  $C_{34}H_{46}O_{10}$ , as stated by Faust.

Lately Ishizu and Kadono (6) have isolated a substance with a digitalis-like action from the Japanese common toad, which they have named "Gamain" and which they have studied by pharmacological methods. However they have been unable as yet to isolate "Gamain" in a chemically pure form.

Five or six years ago Drs. Sanno and Irokawa of our institute made repeated attempts to isolate the active principles of "Sensō," but circumstances unconnected with the problem obliged them to abandon the undertaking. This led me to take up the study of this venom under the direction of Professor Hayashi. With material that had been dried in a calcium chloride desiccator I began my work by repeating the investigations of my predecessors. During the course of the work, the interesting discovery was made by me of the existence in "Sensō" of at least four principles, two of which have been obtained in a state of chemical purity. These together with the two that remain to be further purified will be described in this paper.



## SUBSTANCE A

First some samples of dried "Senso" are ground in a mill to a fine powder (on this occasion one sneezes very violently) which is then extracted by digesting it with warm absolute alcohol. The alcoholic extract is filtered warm and allowed to stand for five or six days, when many lammelar or needle-shaped crystals develop on the bottom of the vessel. The crystals are washed with cold alcohol and allowed to dry in vacuo.

It was observed that this product possesses a digitalis-like action on the frog's heart. Therefore the product appears to be similar to Faust's bufonine, a substance which resembles cholesterine in many ways. But after repeated purification with hot alcohol, the crystals proved themselves to be entirely inactive in a pharmacological way. This substance can not be differentiated from cholesterine by the use of the following color reactions:

1. Dissolve a small quantity of the substance in a test tube in chloroform and stratify under this solution an equal volume of concentrated sulphuric acid. At the line of contact between the two fluids, a deep red color will soon develop, when the two fluids are mixed by shaking and again allowed to separate, and then it will be found that the chloroform also takes up a fine red color. After that on long standing, the sulphuric acid shows a green fluorescence (Salkowski's reaction).

2. Dissolve a small quantity of the substance in acetic anhydride, then add one drop of concentrated sulphuric acid. There appears immediately, first a violet, then a blue and later a green color (Liebermann-Burchard's reaction).

The substance is easily soluble in carbon disulphide, chloroform and hot alcohol, fairly soluble in ether, but little in cold alcohol, and less in petroleum ether and water.

Its melting point is 138°C. (uncorrected). The substance mixed with cholesterine (Merck), dissolved in chloroform and dried again, still retains this same melting point.

As above described this substance is doubtless a cholesterine. In order to settle this point more surely, combustion analyses

of the substance and of cholesterine (Merck) were made. The following tables give some of my analytic results with specimens of Substance A heated to 100°–110°C. for an hour and then dried in vacuo over sulphuric acid to constancy of weight.

*Substance A.* 0.1240 gram substance gave 0.3799 gram CO<sub>2</sub> and 0.1384 gram H<sub>2</sub>O.

*Cholesterine Merck.* 0.1222 gram substance gave 0.3749 gram CO<sub>2</sub> and 0.1386 gram H<sub>2</sub>O.

	SUBSTANCE A	CHOLESTERINE MERCK	THEORETICAL REQUIRE- MENTS FOR THE FORMULA C <sub>27</sub> H <sub>46</sub> O
C.....	83.57	83.67	83.86
H.....	12.49	12.67	11.99

No substance other than that here described which corresponds with Faust's bufonine could be found in "Senso."

## SUBSTANCE B

### I. CHEMICAL

After separating Substance A, the alcoholic solution is freed of alcohol by distillation under diminished pressure, and the residue is dried thoroughly in a desiccator. The dried residue is repeatedly extracted with chloroform and filtered, the chloroform solutions are then poured drop by drop into an equal quantity of petroleum ether. The white precipitate which now falls out turns into a brownish resinous mass. On standing for a day, the clear supernatant fluid (chloroform and petroleum ether) is siphoned off. The fluid (chloroform and petroleum ether) is again poured into an equal quantity of petroleum ether. After separating the precipitate, this procedure is repeated two or three times. The last clear supernatant fluid (a mixture of chloroform and petroleum ether) is allowed to stand in the ice chest. After about a week, a crystalline mass will develop on the bottom of the vessel. This mass appears at first to be amorphous, but examination with the microscope proves it to be finely crystalline. The mass is recrystallized out of a mixture of chloroform and petroleum ether; at this stage the compound has already at-

tained a high degree of purity. Nevertheless this substance has a tendency to undergo change in that the crystals lose their lustre, and on long standing become entirely amorphous..

The crystallization of this substance is not always easily to be attained, sometimes a wax-like mass mixed with crystals develops in the vessel. In such an event the mass is at first washed with benzol, allowed to dry in vacuo and then ground to a powder. The substance when crystallized is but little soluble in alcohol; when still in the amorphous state, it is readily soluble in alcohol. For this reason the mass is washed with alcohol, when the crystals mixed in it remain undissolved. The substance thus obtained is recrystallized twice out of hot alcohol, when it attains a high degree of purity.

The product is easily soluble in chloroform, acetic anhydride and acetone, but very little in benzol, ether, water or petroleum ether. And it is a neutral compound, insoluble in acids or alkalis.

If a small quantity of the product be dissolved in chloroform and a solution of bromine in chloroform added, not the least diminution in the intensity of the color is to be noted (7). The substance has a bitter taste.

The substance shows the following color reactions:

1. Dissolve a small quantity of it in acetic anhydride, then add one drop of concentrated sulphuric acid; a blue color develops immediately, and later a green color. This color reaction depends on the concentration of the solution, sometimes it is entirely similar to that of cholesterine.

2. Dissolve a small quantity of the substance in chloroform and stratify under this solution an equal quantity of concentrated sulphuric acid. When the two fluids are well mixed by shaking and again allowed to separate, it will be found that the sulphuric acid takes up all of the red color, while the chloroform remains entirely colorless (contrary to cholesterine).

3. Dissolve a small quantity of the substance in a concentrated solution of trichloroacetic acid and warm on the water bath, a beautiful yellowish green color develops at once and the solution shows fluorescence later on long standing.



4. Dissolve a small quantity of the substance in glacial acetic acid, add acetyl chloride and a few granules of fused zinc chloride, and warm for a moment over a small flame. A green color develops in a few seconds, which passes soon into a brownish color.

With ferric chloride the substance gives no color reaction.

On the grounds of the above described physical properties, color reactions and the behavior toward bromine, this substance may be considered to be identical or isomeric with the bufagin of Abel and Macht. The following results of elementary analyses may confirm the fact still better.

The melting point is  $209^{\circ}$ – $210^{\circ}\text{C}$ . (uncorrected), which differs somewhat from that of bufagin, which is stated by Abel and Macht to be  $217$ – $218^{\circ}\text{C}$ .<sup>1</sup>

The material used in the analyses was repeatedly recrystallized from a mixture of chloroform and petroleum ether and dried in vacuo over sulphuric acid to constancy of weight. The substance contains no nitrogen.

*Specimen I.* 0.1276 gram substance gave 0.0936 gram  $\text{H}_2\text{O}$  and  $\text{CO}_2$  (failed).

*Specimen II.* 0.1507 gram substance gave 0.3891 gram  $\text{CO}_2$  and 0.1086 gram  $\text{H}_2\text{O}$ .

*Specimen III.* 0.1241 gram substance gave 0.3235 gram  $\text{CO}_2$  and 0.0925 gram  $\text{H}_2\text{O}$ .

	I	II	III	THEORETICAL REQUIREMENTS FOR THE FORMULA FOR BUFAGIN, $\text{C}_9\text{H}_{12}\text{O}_2$ or $\text{C}_{12}\text{H}_{14}\text{O}_4$
C.....		70.53	71.00	71.01
H.....	8.26	8.06	8.33	7.95

## II. PHARMACOLOGICAL

As above mentioned, this substance is but very sparingly soluble in water. It was therefore dissolved in as small a quan-

<sup>1</sup> It is possible that if our substance is crystallized from water after crystallizing it from alcohol as was done by Abel and Macht a melting point of  $217$ – $218^{\circ}\text{C}$ . may be obtained.



tity of hot alcohol as possible and the solution poured drop by drop into a large volume of physiological salt solution, when a fine emulsion is obtained. The emulsion thus obtained, containing of course a little alcohol, was always employed in my pharmacological studies.

### 1. General action and toxicity

*a. Experiments on the frog.* A dose of the substance injected into a frog produces a change in the heart action only. This action is, on the whole, similar to that of the digitalis glucosides. I shall refer to this point again later.

*b. Experiments on the mouse.* In the mouse a hypodermic administration of the drug produces immediately after the injection a rapid rate of respiration and restlessness. In most instances the animal soon shows incoördination of movements. Finally the animal lies on its side, respiratory movements become gradually shallower and slower, which are, when a small dose is given, always succeeded by a recovery. But a large dose of the drug causes violent dyspnoea and fatal convulsions, which end without exception in death. On autopsy following immediately after the death, the heart is in all cases found to be standing still.

#### *Mouse ♀ 9 grams. September 5, 1915*

10 <sup>h</sup> 23' a.m.	0.2 cc. of 0.15 per cent emulsion (0.3 mg.) hypodermically.
26'	Trembling and rapid respirations.
35'	Very restless, turning and washing movements.
43'	Lies on its side, dyspnoea.
52'	Convulsion, death.

#### *Mouse ♂ 8 grams. September 5*

11 <sup>h</sup> 00'	0.1 cc. of 0.1 per cent emulsion (0.15 mg.) hypodermically.
03'	Rapid respirations.
10'	Frequent jumping.
11'	Lies on its side, dyspnoea.
13'	Convulsions.
20'	Death in convulsions.

The following table shows all the results of experiments on the mouse.

NUMBER	SEX	BODY WEIGHT IN GRAMS	QUANTITY OF THE DRUG PER 10 GRAM BODY WEIGHT IN MG.	RESULTS
1	♀	9.0	0.33	Death after 29 minutes
2	♀	9.5	0.47	Recovery after 8 hours
3	♂	8.5	0.17	Death after 20 minutes
4	♂	8.0	0.093	Recovery after 2 hours 20 minutes
5	♀	7.5	0.0046	Recovery after 1 hour 41 minutes
6	♂	24.5	0.18	Death after 1 hour 41 minutes
7	♂	15.5	0.19	Death after 15 minutes
8	♂	12.0	0.12	Recovery after 5 hours 14 minutes

*c. Experiments on the rabbit, the dog and the cat.* In rabbits which receive injections in the ear-vein, the drug causes, on the whole, similar phenomena to those seen in the mouse. Immediately after the administration, rapid respiration appears, the animal lies on its side, showing violent dyspnoea. Finally epileptiform convulsions which end in death are observed.

In the dog and the cat, when administered hypodermically or through the mouth, the drug causes after a short time, nausea and vomiting, accompanied by rapid respirations. The respiratory movements are more vigorous in the last stage of the drug's action, when they are very deep, powerful and of a marked abdominal type, and finally assume the character of the Cheyne-Stokes' respiration. At last, fatal clonic convulsions appear as a rule, and these may possibly be due to the insufficiency of the circulation. On autopsy following immediately after death, the heart is found contracted in systole. A few protocols will illustrate the above noted facts.

*Cat ♀ 1300 grams. October 10*

8<sup>h</sup>58' a.m. 0.7 cc. of 0.3 per cent emulsion (2.1 mg.) hypodermically.  
 9<sup>h</sup>05' Restless.  
 08' Frequent voiding of urine.  
 09' Vomiting, salivation.

12'	Vomiting, ataxic gait, dyspnoea.
21'	Lies on its side, deep and powerful respirations.
28'	Vomiting.
30'	Respiration slower, assumes a Cheyne-Stokes' type.
38'	Death after convulsions.

*Dog ♀ 2350 grams. October 11*

10 <sup>h</sup> 03'	1.0 cc. of 0.3 per cent emulsion (3 mg.) hypodermically.
25'	Frequent urination.
26'	Vomiting.
27'	Vomits frequently.
40'	Lies on its side; dyspnoea.
43'	Cheyne-Stokes' breathing.
50'	Convulsions.
12 <sup>h</sup> 04'	Death.

## *2. Action on the heart*

It is evident, as a result of my observations that this substance is a heart poison, similar to if not identical with those that have already been described as present in the venom of toads. However, in warm-blooded animals, some respiratory disturbances and stimulating phenomena from the side of the central nervous system may in all cases be observed, besides the effects on the heart. With regard to these respiratory disturbances it is impossible for me to determine whether they are due only to the disturbance of the circulation, or rather to a primary action on the respiratory centre itself. Therefore special illustrations of the changes of the respiration are here omitted.

The action of the drug on the heart differs on the whole not so much from that of the glucosides of the digitalis group, but from a few points of view it is not identical with those. From the results of work with the suspension method on the frog's heart, of experiments on the isolated heart of the frog and warm-blooded animals, and from the effects on the blood-pressure of the same, it may possibly be concluded as follows:

1. The lengthening of the diastolic period is not significant. If it does occur, it continues but for a short time.

2. The slowing of the beating of the heart in the therapeutic stage is only of short duration.

3. At the same time, with the appearance of any effect a demonstrable "peristalsis" of the ventricle (in the frog's heart) appears in almost all cases. It passes soon into a toxic stage. Under certain circumstances, the ventricular "peristalsis" may occur prior to the therapeutic stage.

On the grounds above mentioned, I believe that the pharmacological action of the drug supplies no indications for its therapeutic use. It is therefore doubtless that the clinical administration of the drug may be followed by certain dangers.

*a. Effects on the frog's heart in situ.* Varying amounts of the emulsion injected into the subcutaneous lymph sac of the frog, produce several symptoms, somewhat unlike those of the digitalis glucosides as above described, finally a systolic standstill of the ventricle follows. However the ventricular muscle still responds to stimulation. The ventricular "peristalsis" following the therapeutic stage, is observed without exception, if strong well-nourished frogs' hearts be employed; with weak hearts it does not often appear and passes soon into a systolic standstill.

In experiments on frogs I have always employed *Rana esculenta*, *Rana temporaria* in our country, especially in Tokyo, not being suitable for experiments with a heart poison according to Prof. D. Takahashi.

September 4. Room-temperature 21°C.

NUMBER	SEX	BODY WEIGHT (GRAM)	INJECTED QUANTITY OF THE DRUG (MG.)	TIME TILL INCREASING DIASTOLE AND SYSTOLE	TIME TILL THE FIRST "PERISTALSIS"	TIME TILL SYSTOLIC STANDSTILL
				min.	min.	min.
1	♂	27	0.9	5	9	10
2	♂	43.5	0.6	8	10	18
3	♀	49	0.9		2	8
4	♀	49	0.5	4	6	10
5	♂	20	0.3	2	3	5
6	♂	21.5	0.3	2	3	6
7	♂	21.5	0.3	2	4	7
8	♂	15.5	0.15	4	5	10
9	♂	23.5	0.15	2	9	9
10	♂	20.5	0.07	4	12	12



It will be seen from the above table that a marked "peristalsis" seems to appear shortly after the increasing diastole and systole, but when the movements of the heart are registered by a tracing, contemporary appearances of both will be discovered, as will be noted below.

*b. Suspension method of using the frog's heart.* On the other hand, the ventricular, as well as auricular tracings were registered by means of the suspension method, by which the therapeutic stage accompanying a marked "peristalsis" of the ventricle and the heart block, occasionally, is observed.

*Rana esculenta* ♂ 17.5 grams. October 4

TIME	VENTRICULAR BEATS IN 30 SECONDS	ARTICULAR BEATS IN 30 SECONDS	REMARKS
4 <sup>h</sup> 00'	23	23	
01'	23	23	
02'			0.1 cc. of 0.1% emulsion into the lymph sac
05'	23	23	
08'	23	23	
12'	23	23	
13'	23	23	Already "peristalsis"
15'	12	12	Increase of diastole and systole, slowing of beats
17'	12	12	Increase of diastole and systole, slowing of beats
18'	10	10	Beating weakened
20'	8	8	Beating weakened
23'	12	12	Very irregular
25'	11	11	
26'	9	9	
29'	7		
32'	6	6	Beating very little
38'	7	7	
50'			Standstill

1 sec.



A

Before the injection.



B

16 minutes after the injection.



C

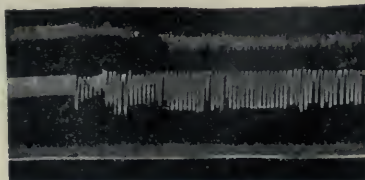
20 minutes  
after the  
injection.*Rana esculenta* ♂ 28.5 grams. October 5

TIME	VENTRICULAR BEATS IN 30 SECONDS	ARTICULAR BEATS IN 30 SECONDS	REMARKS
0 <sup>h</sup> 30'	39	39	
31'	33	33	
32'	33	33	
33'			
35'	31	31	
36'	31	31	
45'	29	29	
47'			
48'	15	15	0.1 cc. of 0.1 per cent emulsion
49'	14	14	Marked "peristalsis"
			Increase of diastole and systole, slow- ing of beats
50'	12	12	
51'	8	8	Increase of diastole and systole, slow- ing of beats
52'	16	16	Irregular
55'	18	18	Beating weakened
57'	16	16	
1 <sup>h</sup> 05'	10	10	
08'	8	8	
40'			Standstill

1 sec.



A

Before the  
injection.

B

14 minutes after the injection.



C

17 minutes after the  
injection.

*c. Action on the isolated frog's heart.* All of my experiments were carried out in accordance with Williams' method. Perfusion with an aerated Ringer's solution containing a small dose of the poison causes a slowing of the heart-beat and an increase of the pulse-volume. In most cases these phenomena are preceded or accompanied by a "peristalsis" of the ventricle. If a somewhat concentrated solution be used the toxic stage appears soon after the "peristalsis," and in no case is an increase of the pulse-volume to be seen. Dilutions as low as 0.0005 percent show an effect on the isolated heart.

*Rana esculenta* ♂ of medium size. November 2

TIME	HEART-BEATS IN 30 SECONDS	PULSE-VOLUME IN CC.	REMARKS
1 <sup>h</sup> 09'	17	0.4	Ringer's solution containing 0.0025 per cent poison perfused
11'	17	0.4	
13'			
14'	17	0.4	
15'	19		Again non-poisonous Ringer's solution perfused
16'			
17'	11	0.6	"Peristalsis" together with an increase of the pulse-volume
18'	11	0.7	
19'	6	0.8	
20'	4	0.8	
25'	2	0.8	
27'	5	0.8	
29'	5	0.8	Slow beats
30'			

Experiment interrupted.



A

Before the perfusion.



B

4 minutes after the perfusion.



C

7 minutes after the perfusion.



D

Somewhat recovered.

*Rana esculenta* ♂ of medium size. November 2

TIME	HEART-BEATS IN 30 SECONDS	PULSE-VOLUME IN CC.	REMARKS
10 <sup>h</sup> 20'	22	0.3	Ringer's solution containing 0.0025 per cent poison perfused "Peristalsis"
23'	22	0.3	
24'			
25'			
26'			Again non-poisonous Ringer perfused
27'			Marked "peristalsis"
28'	12	0.7	Pulse-volume increased
29'	10	0.7	
31'			Beats weak
33'			Very irregular
35'	15	0.4	
37'	8	0.3	
40'	7	0.3	
43'	10	0.3	Somewhat recovered
45'	11	0.3	
48'	10	0.3	
55'	9	0.3	

Experiment interrupted.



*Rana esculenta*, large. October 31

TIME	HEART-BEATS IN 30 SECONDS	PULSE-VOLUME IN CC.	REMARKS
2 <sup>h</sup> 40'	26	0.2	
41'	26	0.2	
42'	26	0.2	
43'			Ringer's solution containing 0.01 per cent poison
44'	25	0.2	
45'	17	0.6	"Peristalsis"
46'			Again non-poisonous Ringer
47'	12	0.6	
48'	10	0.6	
50'	6	0.2	
52'	2		
53'			Standstill

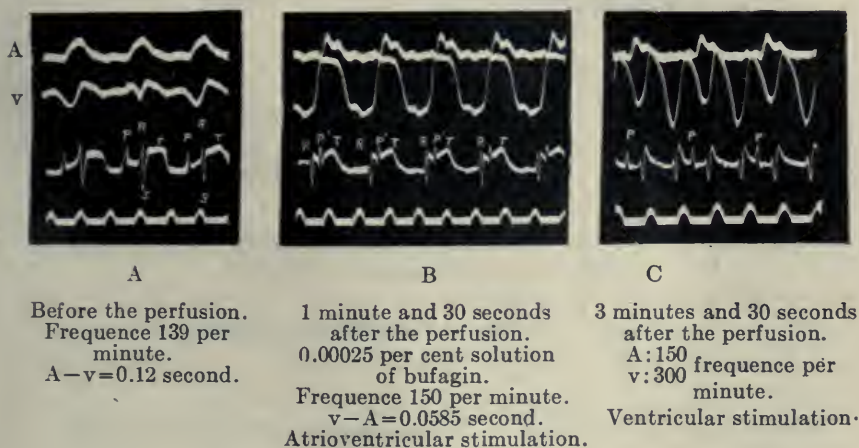
*d. Action on the isolated mammalian heart.* Hearts of rabbits and of dogs were used for experiments which were performed in accordance with Langendorff's perfusion method, oxygenated Locke's solution being the medium employed. In these experiments, a slowing of the heart was not seen; increase of tonicity and of heart rate appear as a rule. The effect here is somewhat similar to that of digitalis on isolated mammalian hearts. Finally the beating becomes irregular and gradually weaker; a true heart block may in almost all cases be observed. It is very probable that the slowing of the heart-beats which is observed in hearts *in situ*, but not in isolated ones has its causation mainly in a stimulation of the vagus-centre by this poison.

The action of the drug on the heart has also been studied for me, by means of the electrocardiogram, by Dr. Shigeru Sakai, in Professor Aoyama's clinic, to whom I here tender my thanks for his kindness.

The electrocardiographic investigations on hearts *in situ*, as well as on isolated hearts, show that differences exist between the action of this drug and that of the digitalis glucosides; though by the usual perfusion of the isolated heart these differences were not apparent.

Under the influence of the drug there seems to be first an increased production of stimuli at Lawara's node, so that both the

auricular and ventricular contraction rates are heightened. Stimulus production in the ventricular node then becomes dominant and the tonicity and rate of the ventricular contractions are evidently increased, while at the same time the auricles resume a normal rate. These ventricular contractions are not regular in rhythm, showing in most cases an extrasystolic type of arrhythmia. Only in the last stage does the sinus node become dominant; the auricular rate then becomes very rapid, while the ventricles contract weakly and slowly, and finally stop in systole. Slow auricular contractions persist for some time. These phenomena are readily observed even when the drug is used in a dilution of 0.00004 per cent. A more detailed report of these experiments will be made in the near future.



### 3. *Effects on the blood-pressure*

Tracings were made of the carotid blood-pressure in rabbits, dogs, and cats under urethane anaesthesia, or after the administration of curare (artificial respiration). Immediately after intravenous injection of the drug there is a rise in blood-pressure, although this is of short duration. The pressure then falls to the original level before the administration, and tends to become somewhat lower. If a large dose of the drug be given, the

preliminary rise in pressure is accompanied by an irregularity of the pulse and the secondary fall in pressure ends in stoppage of the heart. In rabbits a marked rise in pressure was observed, which was either preceded or accompanied by an irregularity of the pulse (no vagus effect); the phenomena being essentially the same as those studied with the frog's heart. Preliminary cutting of the vagi does not serve to prevent the occurrence of the irregularity. During the rise in blood-pressure the respirations are increased in rate and amplitude; later they become gradually slower and slower.

*Rabbit ♂ 2430 grams. October 15. Manometer connected to right carotid artery. injection canula in left external jugular vein; urethane anaesthesia*

TIME	PULSE RATE IN 10 SECONDS	BLOOD PRESSURE IN MM. HG.	REMARKS
8 <sup>h</sup> 49'	39	94	0.3 cc. of 0.3 per cent emulsion injected
51'	40	92	
52'			
53'	38	116	
54'	35	110	
55'	40	100	
56'	36	102	
58'			Again 0.4 cc. of 0.3 per cent emulsion given
58'50"	20	110	Pulse slow and irregular
9 <sup>h</sup> 00'	26	110	
01'	29	142	
02'	30	132	
07'	32	106	
09'			Compression of the abdominal aorta
09'04"		114	Rise of pressure slight
13'	19	94	

Experiment interrupted.

*Rabbit ♀ 2600 grams. October 18. Treated as above; both vagi previously cut*

TIME	PULSE RATE IN 10 SECONDS	BLOOD PRESSURE IN MM. HG.	REMARKS
9 <sup>h</sup> 37'	37	100	Both vagi cut 0.5 cc. of 0.3 per cent emulsion given
38'			
45'			
47'	33	122	
50'	32	118	Again 0.7 cc. of 0.3 per cent emulsion given
54'	32	116	
56'			
57'	32	126	
58'	30	130	1.0 cc. of 0.3 per cent emulsion given Slow and irregular
10 <sup>h</sup> 02'	31	144	
10'	31	140	
12'			
13'	14	128	Pressure has fallen and pulse rate has increased
14'	35	168	
15'	20	152	
22'	41	96	

Experiment interrupted.

*Cat ♀ 1780 grams. October 16. Urethane; manometer connected to left carotid artery;  
injection canula in right jugular vein*

TIME	PULSE RATE IN 10 SECONDS	BLOOD PRESSURE IN MM. HG.	REMARKS
1 <sup>h</sup> 25'	22	90	0.3 cc. of 0.3 per cent emulsion given
30'	33	88	
30'16"	21	104	
30'28"	21	126	
30'40"	19	126	Pulse slow and irregular
31'	20	104	
32'	20	92	
33'	17	84	
35'	17	78	Somewhat recovered
47'	22	78	
48'	21	80	

Experiment interrupted.



*Dog ♂ 2000 grams. October 16. Curare; artificial respiration; in spite of a fall of pressure the experiment was continued in order to observe the effect in such a case*

TIME	PULSE RATE IN 10 SECONDS	BLOOD PRESSURE IN MM. HG.	REMARKS
9 <sup>h</sup> 34'	14	10	
35'	12	14	0.4 cc. of 0.3 per cent emulsion given
37'	13	26	
38'	18	66	Heart action somewhat irregular
39'	19	104	
41'	17	100	
42'	10	50	
43'			Standstill of heart

#### *4. Action on the peripheral blood-vessels*

Experiments on the blood-vessels of both cold-blooded and warm-blooded animals were carried out.

Since large frogs, such as are seen in Europe, are not obtainable in this country, I was obliged to resort to the use of toads [*Bufo japonicus* (Schlegel)]. By means of the Laewen (8)-Trendelenburg (9) method the drops flowing from a cannula in the abdominal vein were collected, and the number of drops per minute counted as also measured in terms of cubic centimeters per minute. The action of the drug on the blood-vessels of the lower extremities of rabbits was also studied. Small, adult, male rabbits were used. Under ether-anaesthesia the abdomen was opened and cannulae inserted in the abdominal aorta and in the inferior vena cava. Warm physiological salt solution is allowed to flow into the artery until the blood of the extremities is thoroughly washed out and the return flow from the vein has become almost clear. Then the body of the rabbit is transversely cut in two at the level of the diaphragm, and the spinal cord immediately destroyed with a wire.

The preparation thus obtained is placed in a thermostat. During the course of the experiment warm oxygenated Locke's solution under a constant pressure is infused through the aortic cannula and the number of drops, and quantity in cubic centi-

*Toad ♂. October 30*

TIME	NUMBER OF DROPS	QUANTITY CC.	REMARKS
11 <sup>h</sup> 01'-02'	50	1.4	1 cc. of a 0.05 per cent solution
06'-07'	52	1.5	
08'-09'	52	1.5	
10'-11'			
11'-12'	47	1.3	
12'-13'	48	1.3	
13'-14'	50	1.4	
15'-16'	52	1.5	
16'-17'	52	1.5	
19'-20'	52	1.5	
21'-22'			0.9 cc. of 0.05 per cent solution
23'-24'	52	1.5	
25'-26'	53	1.5	
27'-28'	54	1.5	
33'-34'	56	1.6	
12 <sup>h</sup> 27'-28'	51	1.3	
29'-30'	51	1.4	

Experiment interrupted.

*Toad ♀. October 18*

TIME	NUMBER	QUANTITY CC.	REMARKS
4 <sup>h</sup> 15'-16'	62	1.7	0.7 cc. of 0.1 per cent solution Slight constriction
17'-18'	60	1.6	
19'-20'			
20'-21'	46	1.3	
21'-22'	51	1.4	
23'-24'	51	1.4	
25'-26'	53	1.4	
33'-34'	53	1.4	
35'-36'			1.0 cc. of 0.3 per cent solution Constriction
36'-37'	27	0.7	
38'-39'	32	0.8	
39'-40'	35	0.9	
41'-42'	36	0.9	
43'-44'	34	0.8	
45'-46'	35	0.9	
55'-56'	35	0.9	

Experiment interrupted.

*Rabbit ♀ 2880 grams. October 28*

TIME	NUMBER	QUANTITY CC.	REMARKS
3 <sup>h</sup> 20'-21'	35	1.3	0.9 cc. of 0.05 per cent solution.
21'-22'	34	1.3	
27'-28'			
28'-29'	31	1.2	
30'-31'	32	1.2	
31'-32'	33	1.3	
32'-33'	33	1.3	
49'			Pressure altered.
51'-52'	46	1.8	
53'-54'	46	1.8	1 cc. of 0.1 per cent solution. Slight constriction.
54'-55'	45	1.7	
56'-57'			
57'-58'	36	1.4	
59'-60'	43	1.5	
4 <sup>h</sup> 00'-01'	44	1.5	
05'-06'	41	1.5	
06'-07'	40	1.5	

Experiment interrupted.

meters, per minute, flowing from the cannula in the vena cava, are measured.

My experiments with these preparations show that the drug produces no remarkable vaso-constriction, or at any rate no decrease in the numbers of drops or quantity in cubic centimeters per minute, unless a very concentrated solution of the poison be used. From this it may be inferred that a rise in blood-pressure produced by administration of a moderate dose of the drug is not due to peripheral vaso-constriction but is associated with increased tonicity of the heart.

### 5. Diuretic action

The diuretic action of the drug was studied on rabbits. In accordance with the method described by Dr. Nukada (10) and his collaborators of this institute, a small incision was made in the lower part of the abdominal wall and through this a cannula

was inserted into each ureter. The number of drops of urine escaping from the cannulae was recorded and simultaneously the blood-pressure of the carotid artery was recorded in the usual manner. Occasionally there is bleeding from a ureter and in such a case the flow from that side is disregarded. My experiments show that the drug causes an increased flow of urine which is in almost all cases accompanied by a rise in blood-pressure.

*Rabbit ♂ 3000 grams. October 25 ✓*

TIME	URINE DROPS		BLOOD PRESSURE IN MM. HG.	REMARKS
	Right	Left		
	In 5 minutes			
1 <sup>h</sup> 20'-25'	12	12	102	0.4 cc. of 0.3 per cent emulsion intravenously.
27'-32'	11	12	100	
34'				
36'-41'	39	32	130	
			118	Trembling.
			116	
43'				
44'-49'	16	17	114	
50'-55'	18	16	114	Again 0.4 cc. of 0.3 per cent emulsion given.
55'-2 <sup>h</sup>	13	12	112	
2 <sup>h</sup> 03'-08'	9	8	114	
09'-14'	6	6	110	
16'				
17'-22'	8	8	134	
23'-28'	7	6	110	
45'-50'	6	6	100	



Rabbit. 2900 grams. October 25

TIME	URINE DROPS		BLOOD PRESSURE IN MM. HG.	REMARKS
	Right	Left		
	In 5 minutes			
2 <sup>h</sup> 04'-09'	Bleeding; of no use	15	108	1 cc. of 0.1 per cent solution intravenously.
10'-15'		12	112	
25'				
25'-30'		17	144	
31'-36'		17	118	
37'-42'		12	112	
44'-49'		9	112	
51'-55'		11	118	
3 <sup>h</sup> 08'-13'		9	116	
18'-23'		11	114	
25'-30'		11	114	1.5 cc. of 0.1 per cent solution.
47'				
50'-55'		19	122	
55'-4 <sup>h</sup>		13	104	
4 <sup>h</sup> 01'-06'		12	100	
07'-12'		11	108	
15'-20'		7	118	

## 6. Haemolysis

With an emulsion of red blood-corpuscles of the rabbit and the dog, no haemolytic action of the drug was observed.

## SUBSTANCE C

## I. CHEMICAL

The residue freed of the Substance A and the Substance B is repeatedly exhausted with benzol. The united benzol solutions are evaporated on the water bath under diminished pressure and the residue is dissolved in ether. When the ethereal solution is evaporated, a yellowish malt-extract-like mass, with a characteristic odor is left. This extract is dried completely *in vacuo* and again treated with benzol and ether as above described. Again dried, a yellowish amorphous substance is obtained. As

this substance is possessed of a characteristic action as will be noted later, I have undertaken to isolate it in a state of chemical purity.

It is readily soluble in almost all organic solvents and gives no precipitate with salts of the heavy metals. My attempts to acetylate it were also futile.

Finally, in order to benzoylate the product it was dissolved in pyridine and into this solution benzoyl chloride, freshly distilled, was allowed to fall drop by drop. After cooling, the whole was poured into a large quantity of water. The red colored oil-like precipitate thus obtained was washed repeatedly with water on a filter-paper. The benzoate thus purified was saponified by means of alcoholic caustic potash and the saponified material was shaken out with ether in a separating funnel. On evaporating the ether, a yellow syrupy substance was obtained, which however was devoid of all characteristic pharmacological action, contrary to my expectation. The Substance C seems, therefore, to have undergone some change in the course of the manipulation. According to this observation then, benzoylating is unsuitable for isolating this substance. And all other attempts to crystallize or to isolate it, even in the amorphous state from several organic solvents have thus far failed.

At last I was obliged to use a product prepared as follows: The crude yellow colored venom was two or three times dissolved in benzol and the residue obtained after evaporating the benzol was washed repeatedly with petroleum ether and set aside to dry. The finely powdered product was then dissolved in alcohol and poured into a large quantity of hot water. The white precipitate that fell out was gathered on a filter-paper, again washed with hot water and dried in vacuo.

As this substance, although chemically impure, has a characteristic pharmacological action, it has been provisionally named "bufotoxine." As thus obtained, bufotoxine softens at 78°C., melts at 83°-85°C. and contains no nitrogen.

## II. PHARMACOLOGICAL

*1. Local action*

Bufotoxine placed on the tongue causes after five or six minutes an insensitiveness which continues for at least three hours or even more. Smeared on the conjunctiva of a rabbit and dog it caused disappearance of the corneal reflex, without previous stimulation. From these facts it is concluded that bufotoxine possesses an anaesthetic action on the sensory nerve endings.

*2. General action and toxicity*

The general effects of bufotoxine after its absorption make it evident that it is a convulsant poison which is to be classed as a member of the picrotoxine group. In several animals, the drug, either injected or given by mouth, produces at first dyspnoea and then, as a rule, violent spontaneous convulsions (a most striking effect) which continue throughout the whole course of the action to death which is probably due to asphyxia as will be shown later.

The convulsant action of the drug evidently differs from that of the strychnine group in its origin. In a frog, in which the connection between the spinal cord and the medulla oblongata was entirely destroyed by means of Baglioni's instrument, the drug, injected into a lymph sac, no longer produces convulsions. On the other hand, a rabbit, whose thoracic cord was completely severed responds with convulsions only in the upper part of the body. But in decerebrate frogs, the drug causes typical convulsions such as are usually seen after the administration of picrotoxine. From this it is easily concluded that the drug acts directly upon the medulla oblongata. And this convulsant action is really typical in dogs and also in hens, the latter of which animals are said to be immune, relatively or completely, to some poisons of the strychnine group.

The skeletal muscle and the motor nerve endings are quite intact, so that the muscle still responds well to either direct or indirect faradisation even after death. One of two muscle-nerve

preparations from a frog, immersed in a Ringer's solution containing the poison, responds as well to faradisation, even after long standing, as the other preparation immersed in a non-poisonous Ringer's solution.

*a. Experiments on the frog.* Injected into a lymph sac the drug causes gradual irritability and finally standstill of the respirations; then follows a series of manifestations of hyperexcitation, beginning with spontaneous spasms in which the flexors have a dominant share, and finishing up by a more or less complete extensor tetanus of the strychnine type. The type of spasms is, on the whole, similar to that produced by picrotoxine, showing a right angle in each joint of the hind extremities and with opisthotonos, etc.; depression and death finally supervene. This action is especially manifest in decerebrated frogs, being well illustrated by the removal of an inhibition from the cerebrum to the so-called convulsant centre in the medulla oblongata. It is easily imaginable from these experiments on frogs alone, that the drug acts directly upon the centre of the medulla oblongata.

*Rana esculenta, medium size. August 6*

- 10<sup>h</sup>43' 0.4 cc. of 1 per cent emulsion injected into the lymph sac.
- 48' The animal opens its mouth often; stoppage of respiration.
- 50' Typical flexor convulsions, opisthotonos.
- 51' Partially paralyzed.
- 52' Strong convulsions.
- 54' Death.

*Rana esculenta, medium size. August 6*

- 11<sup>h</sup>25' At first the left iliac artery is ligated. 0.3 cc. of 1 per cent emulsion given.
- 28' Strong convulsions of both hind extremities.
- 30' Death.

*b. Experiments on the mouse.* The drug injected subcutaneously into the mouse, produces increased rate of respiration, immediately after the injection, washing movements, and finally the animal lies on its side, showing dyspnoea; then follow strong



clonic convulsions with exophthalmus, which end in death from asphyxia. When the chest of the animal is opened immediately after its apparent death, the heart is found still beating weakly.

*Mouse ♀ 14.6 grams. August 9*

- 2<sup>h</sup>24' 0.2 cc. of 1 per cent emulsion subcutaneously.  
 36' Rapid respirations, washing movements.  
 48' Lies on its side, dyspnoeic.  
 43' Strong clonic convulsions.  
 46' Convulsions.  
 3<sup>h</sup>02' Still frequent strong convulsions.  
 03' Exophthalmus and death.

*Mouse ♀ 11 grams. August 9*

- 3<sup>h</sup>07' 0.3 cc. of 1 per cent emulsion given.  
 15' Dyspnoea, lies on its side.  
 18' Strong convulsions.  
 22' Extensor spasms frequently.  
 27' Twitching of jaw muscles.  
 30' Death; on opening the chest, the heart is still beating.

The following table illustrates all the results of experiments on mice.

NUMBER	SEX	QUANTITY OF THE DRUG PER 10 GRAM BODY WEIGHT IN MG.	TIME TILL THE FIRST CONVULSION IN MINUTES	RESULTS
1	♀	1.3	19	Death after 41 minutes.
2	♂	2.7	11	Death after 22 minutes.
3	♂	2.0	10	Death after 13 minutes.
4	♀	1.3	11	Death after 16 minutes.
5	♀	0.7	20	Death after 42 minutes.
6	♂	0.4	25	Death after 36 minutes.
7	♂	0.1	"	Living

*c. Experiments on the rabbit, the dog and the hen.* These animals respond generally, after subcutaneous or internal administration of the drug, with epileptiform convulsions, accompanied by nausea and vomiting. In a cock, an administration of the drug caused repeatedly strong convulsions for a long time. A few examples will illustrate the results.

*Rabbit ♂ 2380 grams. August 10*

- 8<sup>h</sup>55' 1 cc. of 1 per cent emulsion injected in the ear-vein.  
 9<sup>h</sup>01' Dyspnoea, opisthotonus, swimming movements, convulsions.  
 03' Death; on opening chest, the heart is still beating.

*Dog ♀ 1950 grams. August 10*

- 2<sup>h</sup>3' 1 cc. of 1 per cent emulsion hypodermically.  
 47' Dyspnoea; voiding urine.  
 3<sup>h</sup>12' 0.5 cc. of 1 per cent emulsion hypodermically.  
 40' Lies on its side.  
 45' Epileptiform convulsions, in the interval respiration slow and deep.  
 4<sup>h</sup>08' Spasms of the jaw muscles.  
 12' Convulsions.  
 20' Spasm of the jaw and neck muscles.  
 50' Tetanus of long duration.  
 8<sup>h</sup>00' Found dead.

*Cock 1300 grams. August 12*

- 8<sup>h</sup>45' 2.5 cc. of 1 per cent emulsion hypodermically.  
 50' Hind extremities flexed, head lowered.  
 54' Lies on its side, convulsions.  
 55' Respiration slow, eyelids shut.  
 57' Frequent strong convulsions.  
 9<sup>h</sup>44' Death.

In order to analyse the nature of the convulsions, a few experiments were made as follows:

1. In a frog, the brain of which was previously removed, 0.4 cc. of 1 per cent emulsion of the drug injected into the lymph sac caused opisthotonus and typical convulsions in the hind extremities, showing a right angle in each joint.

2. In a frog, in which the connection between the spinal cord and the medulla oblongata was severed by Baglioni's instrument, 0.4 cc. of a 1 per cent emulsion on injection produced no typical convulsions in the extremities, but only twitching of the eyelids and of the submaxillary muscles.

3. In a young rabbit (♂ 1350 grams) the thoracic portion of the hose cord was transected with a sharp knife at the height of

the second dorsal-vertebra and which was kept alive by artificial respiration, 1 cc. of 1 per cent emulsion of the drug injected into the ear-vein, produced spasms of the fore extremities, jaw and submaxillary muscles, but none in the lower part of the body. It is therefore evident that the drug is a poison affecting not the spinal cord but the medulla.

### 3. Haemolysis

The haemolytic action of the drug on the red blood-corpuscles of the rabbit and the dog was observed by the use of the following method, the results being negative.

	5 PER CENT EMULSION OF ERYTHRO- CYTES	EMULSION OF BUFOTOXINE (NO ALCOHOL)	PHYSIO- LOGICAL SALT SOLU- TION	DILUTION	RESULTS
	cc.		cc.		
1	1		1	0	—
2	1	1 cc. of 1 per cent E.*		1: 200	—
3	1	1 cc. of 0.5 per cent E.		1: 400	—
4	1	1 cc. of 0.25 per cent E.		1: 800	—
5	1	1 cc. of 0.12 per cent E.		1: 1600	—
					} At room-temperature (29° C.) for 3 hours.
6	1		1	0	—
7	1	1 cc. of 1 per cent E.		1: 200	—
8	1	1 cc. of 0.5 per cent E.		1: 400	—
9	1	1 cc. of 0.25 per cent E.		1: 800	—
10	1	1 cc. of 0.12 per cent E.		1: 1600	—
					} In a hatching-oven (39° C.) for 3 hours.

\* Emulsion.

### SUBSTANCE D

#### I. REACTIONS OF THE AQUEOUS SOLUTION OF "SENSO"

Some "Senso" ground to a fine powder in a mill was rubbed up with water made faintly acid with acetic acid, and allowed to stand over night. The whole was then filtered, the filtrate con-

pletely precipitated by basic lead acetate, and the excess of lead removed by means of hydrogen sulphide. The filtrate freed of hydrogen sulphide gave the following reactions:

(1) With a solution of ferric chloride the solution gave a fine green color.

(2) A dilute alkali added to the solution produced a beautiful pink color.

(3) The filtrate made slightly alkaline by ammonia produced a carmine-red color on the addition of a drop of iodine-potassium iodide solution.

(4) The filtrate reduced Fehling's solution and ammoniacal silver nitrate solution when boiled for a short time.

These reactions show evidently that the filtrate contains adrenaline or an adrenaline-like substance (a pyro-catechin derivative).

## II. BIOLOGICAL EXPERIMENTS

An aqueous solution of "Senso" obtained as above described was used in some biological experiments.

### 1. *Experiments on the frog's eye*

One of two eyeballs of a frog was immersed in a Ringer's solution containing 6 per cent "Senso," and compared with the other which was kept in a non-poisonous Ringer as a control. The pupil of the former was found in a state of maximum dilatation after fourteen minutes, while the latter still remained contracted.

### 2. *Vaso-constriction*

By means of Laewen-Trendelenburg's method the vaso-constriction of the peripheral vessels of the toad, *Bufo japonicus*, was investigated; the results show that an aqueous solution of "Senso" causes an intense vaso-constriction of the peripheral vessels, such as can only be obtained with adrenaline as far as we know.



*Toad. June 20*

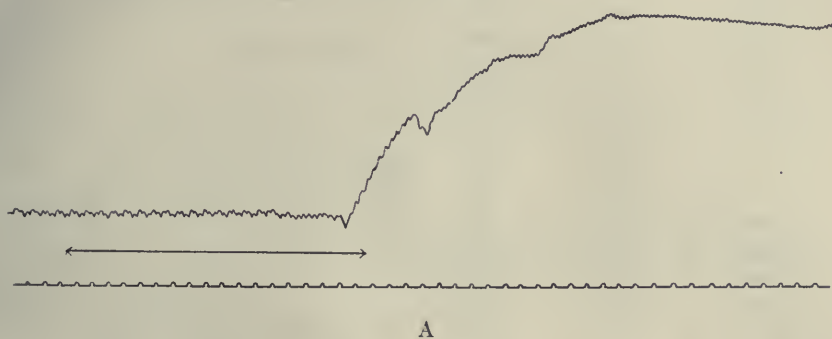
TIME	NUMBER OF DROPS	QUANTITY CC.	REMARKS
0 <sup>h</sup> 35'-36'	48	1.3	1 cc. of 6 per cent solution.
37'-38'	46	1.2	
39'-40'	46	1.2	
41'-42'			
43'-44'	16	0.5	
44'-45'	10	0.3	
46'-47'	10	0.3	
49'-50'	12	0.4	
51'-52'	13	0.5	
55'-56'	16	0.6	
59'-1 <sup>h</sup>	18	0.6	1 cc. of adrenaline (1:100,000).
1 <sup>h</sup> 05'-06'	20	0.6	
10'-11'	22	0.6	
14'-15'	22	0.6	
40'-41'	29	0.8	
42'-43'			
44'-45'	11	0.5	
45'-46'	7	0.2	
51'-52'	7	0.3	
56'-57'	7	0.4	

*Toad. June 20*

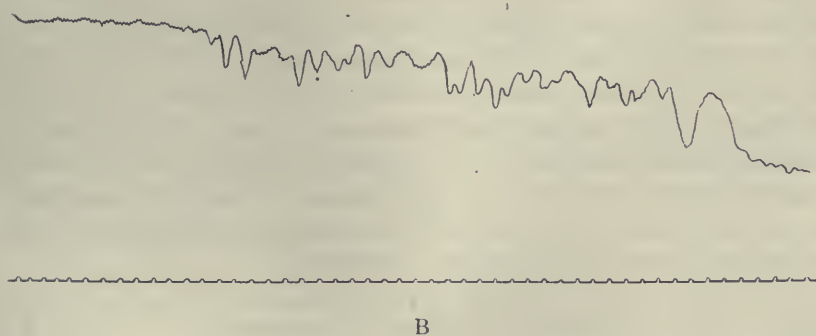
TIME	NUMBER	QUANTITY CC.	REMARKS
3 <sup>h</sup> 07'-08'	48	1.5	1 cc. of adrenaline (1:100,000).
09'-10'	47	1.4	
11'-12'			
12'-13'	7	0.3	
13'-14'	8	0.3	
17'-18'	9	0.3	
21'-22'	13	0.7	
36'-37'	22	0.7	
50'-51'	27	0.9	
54'-55'	27	0.9	
55'-56'			1 cc. of 6 per cent solution of "Senso."
56'-57'	17	0.6	
58'-59'	10	0.4	
59'-4 <sup>h</sup>	8	0.3	
4 <sup>h</sup> 02'-03'	10	0.4	
06'-07'	15	0.5	
11'-12'	22	0.7	
19'-20'	26	0.8	
22'-23'	27	0.9	

*3. Action on the blood-pressure*

In rabbits anaesthetized with urethane the blood-pressure in the carotid artery was measured as usual; the results show that the drug injected into a vein produces a marked rise of blood-pressure, corresponding to its vaso-constrictor action.



Intravenous injection of the substance D.



*Rabbit ♀ 3500 grams. June 5*

TIME	PULSE RATE IN 10 SECONDS	BLOOD PRESSURE IN MM. HG.	REMARKS
2 <sup>h</sup> 22'	35	78	
25'	34	78	
30'			0.25 cc. of 10 per cent solution intra- venously.
30'07"	11	110	
30'20"	24	126	
30'30"	31	188	
30'40"	37	226	
30'50"	28	134	
31'	48	134	
31'30"	43	188	
32'	43	170	
39'	31	120	Slow and irregular.
43'	28	80	Again 0.25 cc. of 10 per cent solution.
43'36"	34	104	
43'46"	44	178	
43'56"	43	208	
44'16"	28	178	
46'	35	134	
47'	29	106	

*4. Hyperglycaemia and glycosuria*

Blood from the ear-vein of rabbits which had previously received subcutaneous injections of an aqueous solution of "Senso," was freed of albumin by means of iron hydroxyde (iron-sol) and its content in blood-sugar was determined by means of an ammoniacal copper sulphate solution in accordance with the Pavy-Kumagawa-Suto method (11). It was observed that the solution of "Senso" produces a high degree of hyperglycaemia in all cases, and that it also induces an accompanying glycosuria as shown by the use of Nylander's reagent.

*Rabbit ♀ 2400 grams. September 7*

9<sup>h</sup>10' 10 cc. blood taken out of the ear-vein, rectal temperature 40°C.; room-temperature 25°C.  
 Blood-sugar, 0.120 per cent.  
 Urine, Nylander (-).

- 11<sup>h</sup>30' 1 cc. of 12 per cent solution of "Sensò" injected hypodermically.  
 12<sup>h</sup>10' 12 cc. blood taken out of the ear-vein, rectal temperature 40°C., room-temperature 26°C.  
 Blood-sugar, 0.200 per cent.  
 Urine, not taken.  
 1<sup>h</sup>35' 8 cc. of blood taken, rectal temperature 40.5°C., room-temperature 26°C.  
 Blood-sugar, 0.368 per cent.  
 Urine, Nylander (+) marked.

*Rabbit ♀ 2900 grams. September 8*

- 10<sup>h</sup>35' 10 cc. of blood taken, rectal temperature 39.6°C., room-temperature 26°C.  
 Blood-sugar, 0.122 per cent.  
 Urine, Nylander (-).  
 11<sup>h</sup>47' 1 cc. of 12 per cent solution injected hypodermically.  
 1<sup>h</sup>50' 10 cc. blood taken, rectal temperature 40°C., room-temperature 27°C.  
 Blood-sugar, 0.313 per cent.  
 Urine, Nylander (-).  
 3<sup>h</sup>10' Urine, Nylander (+) marked.

### III. CHEMICAL

Some pieces of well dried "Sensò" ground to a fine powder in a porcelain (not in an iron) mill, are rubbed up with much water made faintly acid by addition of acetic acid and allowed to stand about forty-eight hours in a dark place. The filtrate is precipitated by basic lead acetate as quickly as possible, and the filtrate from the lead precipitate is immediately treated with hydrogen sulphide. During the course of the manipulation heat was in no case applied.

The clear almost colorless filtrate is condensed to a small quantity in vacuo in a current of carbonic acid on the water bath at 50°-60°C. On the addition of a concentrated solution of ammonia a gray precipitate is obtained which is filtered and washed repeatedly with a solution of ammonia.

The precipitate is again dissolved in dilute acetic acid and ammonia is added. After repeating this procedure, the precipi-



tate is finally dissolved in alcohol containing 2 per cent of pure hydrochloric acid. By adding much ether to the alcoholic solution a small quantity of precipitate develops, which after washing, is immediately set aside to dry in vacuo over pyrogallie acid.

This substance thus obtained is still somewhat of a brownish color, and when dissolved in dilute acetic acid it gives a green color reaction with ferric chloride. Therefore this substance may perhaps be adrenaline; but it remains to be determined whether the substance is entirely identical with the adrenaline contained in the suprarenal gland of the higher animals and in the skin glands of the tropical toad *Bufo agui*, or not. According to Barger and Dale (12), Loewi and Meyer (13), Morita (14), Nagai and Hirose (15), several amine bases possess not only so-called sympathomimetic actions, but some of those derived from pyrocatechin are entirely identical with adrenaline in their biological as well as in their chemical reactions. Therefore further observations must be made in order to settle this point. However; owing to want of the material I am at present unable to proceed further with this investigation but I shall naturally take it up again in the near future when a larger quantity of material will be at my disposal.

#### CONCLUSION AND SUMMARY

1. From "Senso," a Chinese drug derived from toad skins, the author has isolated four principles, named preliminarily Substances A, B, C and D, only two of which, A and B have been obtained in a state of chemical purity.

2. *Substance A*. At first a crystalline substance was easily obtained, which appeared to be similar to Faust's bufonine and showed a digitalis-like action. But the product, after repeated purification, proved itself to be cholesterine, as its melting point and the results of combustion analyses correspond well with those required for cholesterine.

3. *Substance B*. This substance occurs in microscopical crystals when obtained from a solution of chloroform and petroleum ether, and appears to be identical (or isomeric) with the bu-

fagin of Abel and Macht as is proved by its physical properties and color reactions and by the results of the elementary analyses.

The substance has a marked effect on the heart, causes a rise of the blood-pressure and an increased flow of urine, and resembles in its action, on the whole, the digitalis-glucosides. However, it produces in almost all cases a distinct "peristalsis" of the frog's heart, and in the warm-blooded animals the so-called therapeutic stage is of short duration; even with small doses, a toxic stage may easily occur. Therefore this substance is of toxicological interest only, and is quite useless as a therapeutic agent.

4. *Substance C.* This substance, named bufotoxine, possesses a local anaesthetic action, and after absorption its action is that of a medullary convulsant poison. It may be classed with the most efficient members of the picrotoxine group.

5. *Substance D.* The aqueous solution of "Sensu" contains adrenaline or an adrenaline-like substance, which shows several typical color reactions and, biologically, a strong sympathomimetic action. Owing to lack of material the author is at present unable to isolate this substance in a chemically pure state and to proceed further with an investigation of its various properties.

- (1) HAYASHI: Deutsch. med. Wochenschrift, 1911, No. 13, p. 624.
- (2) FAUST: Arch. f. exp. Path. u. Pharm., Bd. 47 and 49. See also by the same author, Die Tierischen Gifte, Braunschweig, 1906.
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- (10) NUKADA and COLLABORATORS: Reports of the Tokyo Medical Society, vol. 28, No. 20, 1914.
- (11) SALKOWSKI: Festschrift, 1904, and HATTA, Reports from the Med. Facult. of the Imperial University in Tokyo, vol. xiii, No. 1, p. 119, 1914.
- (12) BARGER and DALE: Journ. of Physiol., vol. 41, p. 19, 1910.
- (13) LOEWI and MEYER: Arch. f. exp. Path. u. Pharm., Bd. 53, S. 213, 1905.
- (14) MORITA: Arch. f. exp. Path. u. Pharm., Bd. 78, H. 3. u. 4., S. 245, 1915.
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## A CONTRIBUTION TO THE PHARMACOLOGY OF NOVOCAIN<sup>1</sup>

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The toxic actions of cocain resulting from its absorption into the circulation after subcutaneous or intraspinal injection have lead to the introduction into the materia medica of numerous local anesthetics which are claimed to exert relatively slighter systemic effects, but experience has shown that nearly all of the substitutes thus far introduced have greater disadvantages than cocain itself.

Novocain, or the hydrochlorid of para-aminobenzoyl-diethyl-aminoethanol, was introduced about twelve years ago, since which time it has come into extensive use in general surgery and dentistry. The principal advantage claimed for it over cocain is that it is only one-sixth to one-tenth as toxic as the latter, while it exerts a prompt, though fleeting, local anesthetic action.

The literature is extensive, a small volume issued by the manufacturers (Farbwerke vorm. Meister Lucius und Bruening), in 1913, entitled "Novocain" (and from which we shall quote later) contains references to more than five hundred papers, mostly clinical, but the drug has not been the subject of exhaustive pharmacologic investigation, so far as we are aware.

Joh. Biberfeld (Med. Klinik, 1905, p. 1218) reported the results of his investigations of the comparative actions of cocain

<sup>1</sup> This is the second paper from this laboratory dealing with the pharmacology of novocain. The first, "Observations on the Toxicity of Novocain" by J. M. Levy and R. A. Hatcher, appeared in a dental journal, *Items of Interest*, October, 1915, p. 721, which is not readily available to most pharmacologists hence certain data contained in that paper will be used in this one. The protocols of experiments taken from the previous paper will be indicated by asterisks.



and novocain on various animals, from which he concluded that novocain is from five to six times less toxic than cocain by several modes of administration. He did not publish the protocols of his experiments on which these conclusions were based, at that time, and if he has done so since we are not aware of it.

Le Brocq (Br. Med. Jour., March 27, 1909) reported that he had found novocain and cocain to be about equal in anesthetic action, but cocain to be about twice as toxic as novocain for frogs, mice and rabbits.

Petrow (Zent. f. Chir., 1909, vol. 36, p. 482) found that novocain solution diffused rapidly into the tissues after its injection into the veins of portions of the body that had been previously excluded from the circulation, the process of diffusion being practically complete in from five to fifteen minutes. The toxic dose was found to be from two to three times greater, and the fatal dose from seven to ten times greater, when administered in this way than when there had been no previous interference with the entrance of the drug into the general circulation.

Frankfurter and Hirschfeld (Arch. Anat. u. Phys., 1910, p. 515) compared the effects of novocain and cocain on the respiration and circulation in cats after intravenous injection. They report that cocain always alters the action of the heart, but that novocain does not, even when used in doses ten to twenty times as great, though it always causes some fall in blood pressure due to dilatation of the vessels.

Piquand and Dreyfuss (J. de Phys. et de Path. gén., 1910, vol. 12, p. 70) found the fatal intravenous dose of novocain for the rabbit to vary from 42 mgm. to 90 mgm.,<sup>2</sup> dependent on the rate of injection, and cocain to be three and a half times as toxic as novocain under the conditions that he chose for comparison, when injected intravenously, and six times as toxic when the drugs were injected intraperitoneally in 1 per cent solutions.

The toxicity of novocain for man appears to vary even more widely than for the lower animals with a given mode of administration, but in many cases cited in the literature it is difficult to

<sup>2</sup> Throughout this paper doses for animals refer to that for each kilogram of body weight.

determine whether or not the symptoms reported after the administration of very small doses should be attributed to the drug alone.

Liebel (*Muench. med. Woch.*, 1906, no. 5, p. 201) injected a total of 400 mgm. of novocain in 10 per cent solution subcutaneously into himself and an hour later he injected a dose of 750 mgm. in the same manner. The first dose did not induce toxic symptoms, and the total dose, 1.15 grams, produced only mild symptoms of intoxication which lasted about an hour and a half.

Braun (*Erg. de Chir., u. Orthop.*, 1912, vol. 4) used novocain clinically in doses as large as 1.5 grams in solutions of 0.5 to 1 per cent, and states that, except for the occasional vomiting which occurs a few minutes after the injection, side actions were never observed. He says, however, that it is not feasible to state the single maximum dose because the toxicity depends on the mode of administration and the concentration of the solution as much as on the amount of the drug employed.

Siegel (*Med. Klinik*, 1916, vol. 12, p. 34) states that he has used novocain in doses as high as 3 grams in 0.5 percent solution administered in the form of multiple deep injections. In this article he calls attention to the fact that has been mentioned by several observers that dilute solutions of novocain are less toxic than concentrated ones.

In marked contrast to the testimony just cited that tends to show that novocain is relatively non-toxic to man, are reports of severe symptoms of poisoning, and even death, following the use of very small amounts of the drug.

Heineke and Laewen (*Deutsche Zeit. f. Chir.*, 1905, vol. 80, p. 180) observed severe circulatory disturbances with weak pulse and pallor in two cases following the use of doses of 15 mgm. in spinal anesthesia.

Kehr (*Novocain*, p. 205) reported to the Fifth International Dental Congress in Berlin in 1909, that a young woman exhibited symptoms of acute severe cocaine poisoning after the injection of 0.5 cc. of a solution containing 25 mgm. of novocain and 2 drops of solution of adrenalin 1-1000. After two hours the patient suffered an attack of hysteria.

Toxic symptoms of a greater or less degree have been reported after the use of novocain in doses varying from 10 mgm. ( $\frac{1}{6}$  grain) to 130 mgm. (2 grains) by various observers including: Coffart (*Revue de Stom.*, 1911, p. 107); Goodinge and Etheridge (*Br. Med. Jour.*, December 7, 1912, p. 1607); Schlesinger (*Med. Klinik*, 1912, p. 1236); Begg (*Br. Med. Jour.*, February 22, 1913, p. 561); and Giffen and Gundrum (*Calif. State Med. Jour.*, 1914, vol. 12, p. 415). The last mentioned authors review the literature of novocain poisoning briefly, and mention several cases not referred to in the preceding list, and an additional case that was reported to them personally.

Morian (*Zent. f. Chir.*, 1915, vol. 28, p. 493) states that albuminuria was observed by him in a fairly large percentage of his patients who had received novocain.

Vomiting and other minor symptoms are mentioned by many observers as following the use of novocain, as well as other local anesthetics, but it is difficult to determine with any degree of certainty in many of these cases to what extent the drug was responsible for the symptoms. A perusal of the reports mentioned above leaves little doubt, however, in one's mind that novocain was responsible for some of the symptoms, but not for all of them.

So many observers have testified that novocain may be used without serious danger, and such large doses have been used clinically without important toxic symptoms that the dentist or surgeon who has the misfortune to see his patient die after the administration of a dose of the drug which he had reason to consider perfectly safe is apt to fear that he will be blamed too severely if the case is reported. There is good reason, therefore, to believe that fatal poisoning with novocain has not always been reported, and one who has information of two deaths following the use of small doses of novocain writes: "I cannot give you any further information regarding the death of the patients. . . . You can readily understand that a patient dying under the circumstances—that the operator would be loath to publish his experience." We might add that we have not seen either of these cases reported in the literature.



We have made no effort to examine the clinical literature of novocain comprehensively, and have not tried to determine the total number of deaths that have followed its use. The succeeding reports of death following its use have, however, been gathered in a brief survey of the literature and in addition, as previously mentioned, we have been informed of two deaths. In one of these cases the dose did not exceed 20 mgm., and in the other it was probably no larger. In one instance the patient is said to have "rolled off the table in a condition of opisthotonos" immediately after the injection of the drug and to have died of failure of respiration. The details of the other case could not be learned, except that the dose was small.

Gabbett (*Br. Med. Jour.*, 1910, p. 690) reported the death of a man following a slight operation under spinal anesthesia, for which he had administered 100 mgm. of novocain and 1 mgm. of strychnin hydrochlorid. Death resulted from respiratory failure, artificial respiration being rendered difficult by rigidity of the arms and chest.

Scandola (*Gaz. deg. Ospedale*, January 14, 1915; through abstract in *J. A. M. A.*, vol. 64, p. 866) reported the death of a patient following the intraspinal injection of a "small amount" of novocain. It is further stated in the abstract that Merusel had reported the death of two patients following the use of small doses of novocain.

The muscular spasms reported in several of the cases which resulted fatally, and their appearance in animals poisoned with novocain, leave no doubt that the drug was the cause of death in these instances. Gabbett was inclined to blame the strychnin in his case, but such a small amount of strychnin has never been known to cause the death of an adult. Others have suggested that epinephrin caused poisoning in certain of the cases reported in which it had been used with the novocain, but there is no reason to suppose that it contributed to these accidents, especially in view of the results of certain experiments that we shall report later.

The following cases cited by Groede (*Arch. exp. Path. and Pharm.*, 1912, vol. 67, p. 172) show that cocain also exhibits ex-



traordinary variations in the degree of its toxicity for man; according to authors whom Groede quotes death followed the use of cocain in three cases where the doses were 16, 40, and 60 mgm. respectively. On the other hand, he states that many observers have reported the administration of doses of 1 gram of cocain without death resulting and in one case, at least, a dose of 1.25 grams injected subcutaneously was survived. He states that numerous authorities give the fatal dose of cocain for man as being between 22 cg. and 1 gram.

#### EXPERIMENTAL

Cats, dogs and rabbits were used in the earlier series of experiments in this laboratory in which it was sought to determine the fatal dose of novocain, but the effects of the intravenous injection of the drug were essentially similar in the several species of animals, and cats were therefore used almost exclusively in the later studies.

In cats the subcutaneous injection of 100 mgm. of novocain in 10 per cent solution caused nausea and muscular weakness within seven minutes, followed by apparently complete recovery within a period of about an hour. Similar doses of novocain injected subcutaneously together with from 0.2 to 2.5 mgm. of epinephrin caused no perceptible effects.

The low degree of toxicity of novocain for the normal cat by subcutaneous injection, and more especially when combined with epinephrin, is shown by the results of two of the experiments; in one of these a dose of 250 mgm. of novocain was injected alone, and in the other 500 mgm. of novocain were administered with epinephrin without causing death in either case.

*Protocols—Toxicity of novocain by subcutaneous injection.*  
*Cat, weight 3.04 kg.*

- 2.35 p.m.    Injected subcutaneously 250 mgm. novocain per kilogram  
                  in  $33\frac{1}{3}$  per cent solution.  
3.15            Muscular incoördination; legs stiff.

- 3.35 Sits quiet, apparently normal when undisturbed; when dropped from height of 2 feet shows muscular incoördination.
- 5.00 Appears nearly normal; there has been no nausea; eats meat. Following morning animal normal.

*Cat, weight 2.6 kg.*

- 2.37 p.m. Injected subcutaneously 500 mgm. novocain per kilogram in  $33\frac{1}{3}$  per cent solution, containing epinephrin 1-3000.
- 3.15 Some distress.
- 3.17 Violent tonic and clonic convulsions.
- 3.23 Panting; muscular weakness; reflexes normal. Convulsions absent.
- 4.26 Animal unable to sit up.
- 5.00 Marked distress; unable to sit up. Following morning animal appears normal, but does not eat.

The dose of novocain which was survived by the cat used in the second experiment detailed above was several hundred times larger, relative to the size of the animal, than that which caused the death of one of the patients previously referred to. It did not seem worth while to pursue this phase of the subject further, but it may be mentioned that Heineke and Laewen (loc. cit.) found the fatal dose of novocain for the rabbit by subcutaneous injection to be about 730 mgm. whether it was given alone or in combination with epinephrin.

The smallest fatal intravenous dose of novocain for a normal cat in the series of experiments made in this laboratory was 40 mgm., the injection having been made very rapidly. This dose corresponds closely with that required to kill the rabbit by a similar mode of administration, as determined by Piquand and Dreyfus (loc. cit.).

Much smaller doses, however, caused intense, and even threatening, symptoms of poisoning, and doses of only 15 mgm. produced severe disturbances of the circulation and respiration in cats and rabbits.

The effects of the rapid intravenous injection of all doses that failed to cause death were fleeting, and the condition of the animals

became normal after the lapse of only a few minutes. The protocol of an experiment will serve to illustrate this point.

*Protocol—Toxicity of novocain by rapid intravenous injection*

*Cat, weight 1.74 kg.\**

11.33, 15 a.m.	Injected 25 mgm. novocain per kilogram, in 5 per cent solution.
11.33, 20	Heart beat stopped; animal struggled for about two minutes; prompt recovery.
11.48, 22	Injected 25 mgm. novocain per kilogram as before.
11.48, 27	Symptoms as before.
12.32, 47	Injected 40 mgm. novocain per kilogram as before.
12.32, 52	Heart beat imperceptible.
12.34, 30	Heart beat again perceptible, very irregular.
12.36, 15	Clonic convulsions, respiration stopped.
12.40	Heart beat imperceptible.
	No recovery.

The rapid intravenous injection of a dose of 40 mgm. resulted fatally almost invariably. Attempts were then made to determine the maximum amount of novocain that could be injected intravenously into cats within a period of several hours without causing death.

One animal received the enormous dose of 372.5 mgm. of novocain within a period of three and a half hours, and after an interval of only twenty minutes it received an additional dose of 35.5 mgm. injected rapidly into the vein. This latter dose was about 90 per cent of the amount usually fatal when so injected, but the animal behaved toward it exactly as would a normal animal—in other words, the effects of the enormous amount that had been injected previously had apparently passed off completely, and within five minutes after the injection of the last dose the heart beat and the respiration had become normal and the cat continued to behave in every way like a normal animal.

This animal received a total of 408 mgm. per kilo, by vein, or more than twenty-five times the amount required by rapid in-

jection to cause severe symptoms of poisoning, but no effects of any kind could be observed after the first few minutes following the completion of the administration.

Another cat received a dose of about 400 mgm. intravenously within a period of about three and a half hours, after which its condition became apparently normal within a few minutes, and it showed no effects of the drug subsequently.

These two experiments were essentially similar except for the rapid administration of the last portion of the dose in the first one, and the protocol of only one of them will be given here.

*Protocol—Toxicity of novocain by slow intravenous injection*

*Cat, weight 2.3 kg.\**

- 11.30 a.m. Began injection of novocain in 1 per cent solution.
- 12.45 p.m. Twitching about head, respiration shallow; 150 mgm. per kilogram have been injected.
- 3.00 Cat has received 372.5 mgm. novocain per kilogram; shows great depression; injection stopped.
- 3.20 Injected rapidly 35.5 mgm. per kilogram of novocain in about 1 cc. of solution. Heart beat became imperceptible almost at once; convulsion; respiration ceased; after about one minute the heart beat and respiration returned and improved rapidly.
- 3.24 Animal appears to be about normal.
- 4.24 Animal has eaten food. Following morning, animal normal.

It is surprising to find that the heart and respiration are affected profoundly, but independently, by nearly fatal doses of novocain. Both stop within a few seconds when a moderately large dose of the drug is injected rapidly into the vein, hence the action on the one is independent of that on the other.

The results of these experiments show that the amount of novocain required by intravenous injection to cause death varies very widely with the rate of administration, and that enormous amounts can be injected slowly without causing death.



It is interesting to note that cocain shows an analogous variation in the fatal dose dependent on the rate of intravenous injection, but recovery is not so complete and death results after the administration of a much smaller amount than that of novocain which was survived by animals in this laboratory without lasting effects.

Groede (loc. cit.) cites widely differing results obtained by different investigators who had attempted to determine the fatal dose of cocain for several species of animals by different modes of administration. It is obvious that the rate of administration of cocain plays an important rôle, as is the case with novocain.

The experiment detailed below does not serve to fix the minimum fatal dose of cocain but it does help to explain the discordant results reported by other observers.

*Protocol—Toxicity of cocain by slow intravenous injection*

*Cat, weight 2.2 kg.*

10.39 a.m.	5 mgm. cocain hydrochlorid per kilogram in 1 per cent solution into femoral vein; respiratory embarrassment at once.
10.40	Heart slow and irregular, soon increasing at rate.
10.43	Animal appears to be normal.
10.45	5 mgm. cocain per kilogram as before; symptoms as before plus mydriasis.
10.54	5 mgm. cocain per kilogram as before; symptoms as before mydriasis persisted to end of experiment.
11.04	5 mgm. cocain as previously; symptoms much as before; recovery in interval.
11.16	5 mgm. cocain as previously; symptoms much as before. recovery in interval.
11.25	5 mgm. cocain as previously; symptoms as before plus clonic convulsions.
11.46	5 mgm. cocain as before; symptoms much as before.
12.01 p.m.	5 mgm. cocain as before.
12.46	5 mgm. cocain as before.
1.00	5 mgm. cocain as before; almost constant state of opisthotonos.
1.35	5 mgm. cocain as before; respiration extremely shallow.

- 2.17        5 mgm. cocain as before.  
2.34        5 mgm. cocain as before; animal very weak.  
2.45        5 mgm. cocain as before; respiration extremely shallow, no convulsions.  
2.55        5 mgm. cocain as before; respiration obviously about to fail.  
3.10        Death without further injection of drug.  
*Résumé.* 75 mgm. cocain per kilogram in 15 injections in 4 hours and 16 minutes.

The cat that received the repeated injections of cocain showed severe cardiac and respiratory disturbances after a single injection of 5 mgm. of the drug, but it did not succumb before fifteen such injections had been made at intervals during a period of four hours and sixteen minutes. Another cat died from a much smaller dose injected slowly, and a third died only after receiving a larger dose, though it would probably have died eventually had the injection been stoppped much earlier. This method is evidently of little or no value in the determination of the minimum fatal dose of cocain.

It is obvious from the results here reported that one cannot speak correctly of a fixed ratio of toxicity for novocain and cocain without reference to the mode of administration and to the concentrations of the solutions employed, and furthermore, that the ratio which applies to one mode of administration for one animal does not apply to another mode of administration for the same species, and that the ratio which applies to one mode of administration in one species does not apply to the same mode in another species.

It is a curious fact that little attention has been paid to the statements made by several observers that the toxicity of novocain depends to an extraordinary degree upon the rate at which it enters the blood stream, and different authorities continue to state the ratio of toxicity in fixed terms varying from two to one, as stated by Le Brocq, to about six to one, as stated by Biberfeld.

The statement by Biberfeld has proved especially misleading since it appears to have gained wide acceptance among clinicians

without an appreciation of the true relative toxicity of the two drugs.

We do not believe that anyone, including even those who have called attention to the extraordinary variation in toxicity dependent on the rate of injection, has had any true idea of the real extent of this variation such as is shown by the experiments detailed here, at least no one has given any figures suggesting such variations.

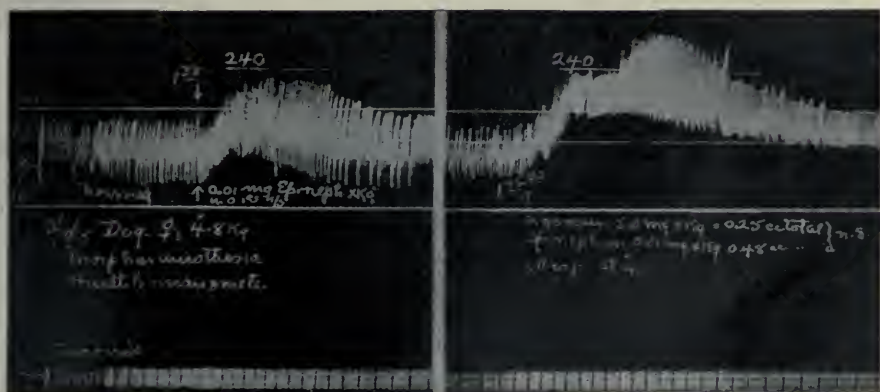
It is also obvious that the determination of the toxicity of cocain and novocain for normal animals affords no satisfactory explanation of the accidents that have occasionally followed the clinical use of very small amounts, but the extraordinarily rapid action that these drugs exert on the heart and respiratory center suggests that such accidents are probably due to some abnormality of the heart or respiratory center whereby the susceptibility to the toxic actions of the drugs is increased to an extraordinary degree.

This view finds support in the results of some of the experiments on cats which had previously received large doses of hydrated chloral and which showed profound respiratory depression. The results in these experiments were, however, far from uniform. One cat survived the rapid intravenous injection of a dose of 30 mgm. of novocain (75 per cent of the fatal dose for a normal animal by this mode of administration) while under the influence of a dose of hydrated chloral sufficient to cause moderate narcosis, but another chloralized animal succumbed to the injection of a dose of only 10 mgm. of novocain similarly administered.

It is at least interesting to note that Levy and Hatcher found that the simultaneous intravenous injection of epinephrin interfered to some extent with the toxic action of novocain. One of their animals survived the rapid injection of a dose of novocain 50 per cent larger than that which is usually fatal in normal animals by that mode of administration, epinephrin having been injected at the same time. The results were not constant but there is an unquestioned antagonism between these two drugs when they are rapidly injected intravenously into cats.



This seems the more remarkable when one learns that these drugs show a synergistic action on the frog's pupil (Biberfeld, loc. cit.) and on the blood vessels, as shown by our own experiments. This synergism between novocain and epinephrin is analogous to that of cocain, and epinephrin whereas while cocain alone stimulates the vaso-constrictor ending, causing a rise of blood pressure, novocain alone causes a fall of blood pressure even in small doses which, according to Frankfurter and Hirschfeld (loc. cit.) do not affect the heart markedly. This synergistic action of novocain and epinephrin is shown in tracing 1, in which the rise of blood pressure is not only higher,



Tracing No. 1 showing rise of blood pressure to about 220 mm. of mercury following the intravenous injection of 0.01 mg. epinephrin per kilogram and a greater rise to about 300 mm. after a similar dose of epinephrin combined with 5.0 mg. novocain per kilogram.

but is also more sustained than after a similar dose of epinephrin alone, while the fall of blood pressure induced by a similar dose of novocain alone is shown in tracing 2.

Piquand and Dreyfuss (loc. cit.) state that mixtures of novocain and epinephrin are not notably more toxic than novocain alone when injected intravenously, but they did not attempt to determine whether the mixture was less toxic. They did, however, observe the lessened toxicity of the mixture by intraperitoneal injection and seemed to think that their observations



appeared paradoxical at first sight. However, the vasoconstrictor action of epinephrin is almost universally utilized to retard the absorption of certain local anesthetics and the subject therefore hardly deserves further discussion here.

There are few non-volatile drugs that produce such profound, but fleeting, intoxication as that described here for novocain, and we next undertook to discover the reason for the phenomenon. Several possible explanations suggest themselves: (1) The drug might exert its toxic action only when in certain concentration and its rapid dilution in the blood stream might account for the prompt disappearance of the symptoms. (2) The concentration in the blood stream might be diminished through



Tracing No. 2 showing fall of blood pressure immediately following the intravenous injection of 5.0 mg. of novocain per kilogram.

rapid diffusion into the body tissues, as suggested by Petrow (*loc. cit.*). (3) The drug might be excreted rapidly. (4) It might undergo fixation or destruction in some organ.

Against the first hypothesis (mere dilution in the blood stream) is the fact that very shortly after the completion of the slow administration of an amount equal to several times the fatal dose by rapid injection the animal behaves toward a rapidly injected dose, which would be nearly fatal for a normal animal, exactly as though none of the drug had been given previously. The drug has evidently left the blood stream, or been fixed or decomposed.

Regarding the second hypothesis (rapid diffusion from the blood vessels) it does not seem probable that dilute solutions of

novocain leave the blood stream by mere diffusion so rapidly as would be necessary to explain the phenomenon. It should be recalled that Petrow dealt with concentrated solutions of novocain injected into a vein in which the circulation had been interrupted. In connection with the third hypothesis (rapid excretion) it seemed to us equally improbable that the kidney eliminates the drug so rapidly, at any rate we do not recall that any analogous substance is excreted in the urine at such a rate as novocain would have to be in order to account for the prompt recovery observed by us.

We were therefore inclined to look to the liver as the organ concerned in the fixation, elimination or destruction of the poison. We were perhaps the more inclined to suspect that the liver is concerned with the removal of the drug from the blood stream because we had previously found that the liver of the white rat removed a large part of a nearly fatal dose of ouabain from the blood stream within a period of a few minutes after its intravenous injection. (The details of these experiments will probably be published shortly.)

In order to test whether the blood fixes novocain a dog was bled, 375 mgm. of novocain were added to 100 cc. of the blood, which was then defibrinated and a portion was reinjected into the animal from which it had been withdrawn. The effects of the novocain thus injected were the same as those produced by an equal amount dissolved in saline solution.

An attempt to compare the toxicity of novocain when administered by the portal vein with that induced by injection into the femoral did not give wholly satisfactory results, but the experiments did seem to indicate that the liver exerts some protective action.

In order to determine whether the liver removes or destroys novocain perfusion experiments were undertaken. The livers of two cats were perfused with solutions of known amounts of novocain and the perfused fluid was then tested upon normal cats, because we lacked any suitable means of estimating the amount of novocain present by chemical methods, and because the cat reacts to this drug in a typical manner. The re-

sults of two such experiments leave no doubt that the liver does actually fix or destroy novocain rapidly, and since these two experiments were practically alike the protocol of one will suffice.

*Protocol—Perfusion of cat's liver with novocain solution and blood*

A cat weighing 2.5 kilos was exsanguinated through the carotid artery while about 100 cc. of Locke's solution were being injected into the femoral vein. Two hundred and fifty cubic centimeters of blood (the latter portion diluted somewhat with the injected Locke's solution) were collected and defibrinated in the usual manner and strained.

The liver, weighing 88 grams was washed nearly free of blood by passing Locke's solution through the portal vein. The liver was then removed from the body and perfused at a constant temperature for one hour and twenty minutes with a solution of 500 mgm. of novocain in 200 cc. of the defibrinated blood. The blood was allowed to escape into a beaker in which it was oxygenated and from which it was returned to the perfusion reservoir. It is estimated that the greater part of the blood passed through the liver from five to seven times.

The fluid that was obtained at the completion of the perfusion measured 190 cc. Allowing for diffusion into the tissue fluids of the liver, so that any novocain that escaped fixation or destruction would be distributed uniformly through the fluids we estimated that the 190 cc. of perfused fluid recovered should contain not less than 70 per cent of the novocain originally present, or (supposing that none had been fixed or destroyed) about 1.75 mgm. per cubic centimeter.

The perfused fluid was then tested biologically as follows: Fifty cubic centimeters were injected into the femoral vein of a cat weighing 1 kgm. as rapidly as such a large volume of fluid could be with safety, that is within a period of seven minutes. No perceptible effects were induced.

As previously mentioned, as little as 15 to 20 mgm. was found to cause profound cardiac and respiratory disturbance, hence we must conclude that the 50 cc. of perfused fluid contained much less than 20 mgm. of novocain, or less than one-fourth of the amount that it would have contained had none been lost during the perfusion of the liver. As a matter of fact, we have reason to suppose that even a larger proportion of the novocain was lost during the perfusion.



While the perfusion experiment just detailed serves to show that the liver does actually remove the novocain from the perfused fluid, it does not indicate whether it is merely fixed in the tissues or destroyed. We therefore endeavored to determine whether it could be recovered by simple extraction after the addition of a known amount to the hashed liver, and detected by the biological test.

We devised a method by which we hoped to be able to extract novocain from the tissues, and tested it on blood, after having shown that novocain is not fixed or destroyed in the blood. The extracts thus prepared were tested biologically on cats, the results showing conclusively that novocain could be recovered from its solution in blood with little, or no, loss. Having shown the method to be adequate we used it in the endeavor to determine whether novocain previously added to liver tissue was present in a form that permitted of its recovery. A protocol of an experiment dealing with the blood and one with the liver will be given.

*Protocol showing extraction of novocain from blood*

Two hundred and fifty milligrams of novocain, dissolved in 25 cc. of Locke's solution, were mixed with 38.5 cc. of blood that had been drawn from a cat; 200 cc. of alcohol were added, the mixture stirred for a few minutes and poured on a filter; the residue on the filter was returned to a beaker, mixed with 100 cc. of alcohol and returned to the filter. The mixed filtrates (estimated to contain about 90 per cent of the added novocain, or 225 mgm.) were evaporated on a water bath in a strong current of air to a small volume, a drop or two of acetic acid was added and the mixture heated to precipitate protein, after which the volume was made up to 50 cc. and filtered. Each cubic centimeter of the finished filtered extract should have contained 4.5 mgm. of novocain (supposing none to have been lost in the later steps of the process). The extract was tested biologically as follows:

*Cat, weight 2.24 kg.*

2.55 p.m. Injected 4.1 cc. extract per kilogram into the femoral vein, heart beat impalpable and respiratory disturbance almost immediately; heart and respiration normal within a few minutes.



- 3.01-3.02 Injected 7.3 cc. as before. Symptoms much as in preceding but more intense. Rapid recovery.  
3.07-3.08 Injected 8.5 cc. as before. (38.5 mgm. novocain?) Effects as before. Animal chloroformed.

There is little doubt that the finished extract contained nearly, or quite, the calculated amount of novocain. That there was nothing else in the finished extract to induce the toxic symptoms is shown by the results of a control experiment in which 50 cc. of blood without novocain were extracted and tested in the same way without producing the slightest effect in the test animal.

In one experiment the technic was modified by expressing the mixture of blood and alcohol; the filtrate and the finished extract were turbid and when tested biologically the extract showed a slightly greater toxicity than could be accounted for by the novocain that it was supposed to contain.

*Protocol—Extraction of liver for novocain*

A cat weighing 1.75 kilos was killed, the liver excised and a portion weighing 22 grams (approximately one-third of the organ) was hashed and mixed with 25 cc. of a solution containing 250 mgm. of novocain. This was macerated for two hours at a temperature of 37.5°C. after which it was extracted in the manner already described for the recovery of novocain from the blood. The final filtered extract, which was calculated to contain 4 mgm. of novocain in each cubic centimeter less any that might have been fixed or destroyed by the liver tissue, was tested as follows:

*Cat, weight 1.72 kilos*

- 2.48 to 2.52 p.m. Injected 14 cc. of extract per kilogram into femoral vein. Respiration and heart feeble.  
— to 2.55 Injected 27 cc. extract per kilogram (includes preceding). Respiration failed gradually, heart failed only after one minute.

The failure of this solution to cause the heart to stop immediately points rather strongly to the presence of much less novocain than was estimated that it would contain if none had been removed from the liver tissue.

In one case 200 mgm. of novocain were added to a liver weighing 105 grams; this was hashed and extracted at once. The injection of a total of 30 cc. of the finished extract into the vein of a cat weighing 1.6 kilos in a period of thirteen minutes (22 cc. in the last five minutes) resulted in fairly typical symptoms of novocain poisoning—cardiac and respiratory standstill, with prompt recovery. This points to the destruction of a considerable proportion of the novocain, though not all. The results of the extraction experiments with the liver indicate some destruction of the novocain by that organ, but they are not wholly satisfactory owing to the difficulty of obtaining extracts which are devoid of physiological actions that are attributable to the tissue extracts.

It is probable that little novocain is excreted unchanged in the urine of the cat, at least we failed to obtain evidence of its excretion in the urines of two cats that had each received an intravenous injection of a gram of the drug in a period of 2 to 3 hours. At the conclusion of the experiments the animals were killed, their bladders excised and the urine collected, none having been voided during the experiments. The urines were tested biologically for novocain; since the results were essentially similar in the two experiments the protocol of but one will be given.

*Protocol—Test for novocain in urine*

*Cat, weight 2.92 kilos*

- |               |  |
|---------------|--|
| 11.50 a.m.    | Bladder emptied by manual compression.   |
| 11.54 to 2.40 | Injected intravenously 1 gram novocain—total—in 1 per cent solution.                             |
| 2.50          | Animal killed with chloroform, bladder excised, 19 cc. of urine obtained, and tested as follows: |

*Cat, weight 2.2 kilos*

- |              |   |
|--------------|---|
| 3.01 to 3.03 | Injected intravenously all of urine obtained from preceding cat. No symptom except slight acceleration of the heart rate. |
|--------------|---|

Had as much as 3 per cent of the total novocain injected into the first animal been excreted unchanged in the urine it would have produced severe symptoms of intoxication in the test animal.

#### SUMMARY

It is still commonly stated that the toxicity of novocain is about one-sixth to one-tenth that of cocain, even though several observers have called attention to the dependence of the toxicity of novocain on the mode of administration, and especially on the rapidity which it enters the circulation.

The toxicity of novocain is greatest when a concentrated solution is injected rapidly into the vein, in which case a dose of 40 mgm. per kilo is fatal to the cat and rabbit, and probably to other animals, though much smaller doses cause severe, and even threatening, symptoms. Very much larger doses may be injected slowly into the vein or subcutaneously without causing more than temporary disturbances.

Cocain shows an analogous though slighter variation in toxicity dependent on the mode of administration.

The subcutaneous injection of a mixture of novocain and epinephrin results in greatly delayed absorption and consequently diminished toxicity of the novocain for the cat. When such a mixture is injected intravenously there is a synergistic constrictor action on the vessels, with an antagonistic effect on toxicity probably due to the action of epinephrin on the heart.

The toxicity of novocain is increased, but in a variable degree, by the previous administration of hydrated chloral which depresses the respiratory center.

The extremes of toxicity of novocain shown when it is injected rapidly into the vein of a chloralized cat (10 mgm. per kilogram, fatal) and when administered slowly to a normal cat (408 mgm. per kilogram with only temporary disturbance) suggest a possible explanation of the accidents occasionally seen when small doses of novocain are used clinically.

Novocain leaves the blood stream rapidly, being fixed or destroyed in the liver, the weight of evidence pointing to its destruction in that organ.

Less than 3 per cent (if any) of a large intravenous dose is excreted unchanged in the urine of a cat within a period of two to three hours.

#### CONCLUSIONS

One is not justified in speaking of the ratio of toxicity for cocain and novocain without reference to the mode and rate of administration, the concentration of the solution used and the species of animal employed.

Novocain in concentrated solution may be fatal in smaller doses than cocain if the latter pass slowly into the circulation and the former rapidly. Nevertheless there is no reason to doubt that novocain is safer than cocain when used properly.

The proper clinical use of novocain requires attention to the condition of the heart and respiration, and the avoidance of its rapid entrance into the circulation.

The combination of epinephrin with novocain certainly delays absorption from the subcutaneous tissues, and probably enhances the local anesthetic action of the latter drug.

The liver removes novocain from the circulating blood rapidly and this almost certainly accounts for the prompt return to normal following the rapid intravenous injection of a nearly fatal dose. Little, or none, of the drug is excreted unchanged in the urine of the cat.





# THE INFLUENCE OF ATROPINE AND PILOCARPINE ON THE GLYCOGENIC FUNCTION

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In 1883, Morat<sup>1</sup> published a work on this subject and concluded that pilocarpine stimulates the formation of sugar from glycogen and that atropine has the opposite action. This work is the basis for the statement still carried in textbooks on pharmacology<sup>2</sup> that pilocarpine increases the blood sugar. The acceptance of this statement necessitates the presence of glycosecretory fibres to the liver. From a study of the effects of stimulation of the coeliac plexus before and after the administration of atropine, Cavazzani and Soldaini<sup>3</sup> concluded that atropine paralyzes the glycosecretory fibres to the liver, and Rudisch<sup>4</sup> states that in diabetics, carbohydrate tolerance is increased by atropine sulphate. Forscheimer,<sup>5</sup> in some cases, confirms this statement. Mosenthal<sup>6</sup> and Macleod<sup>7</sup> could find no evidence in support of these statements. Recently, Eiger<sup>8</sup> reports that stimulation of the vagus increases the formation of glycogen in the liver of turtles when perfused with dextrose.

The conclusions of Morat, Cavazzani, Rudisch and Eiger, are based on meagre and unsatisfactory data and in view of Macleod's more convincing work to the contrary, we have thought it advisable to report evidence on this subject which definitely shows that pilocarpine and atropine have very little action on

<sup>1</sup> Morat: Lyon Medical 1883, April, xlii, 545.

<sup>2</sup> Cushny, Wood, Sollman, Dixon, et al.

<sup>3</sup> Cavazzani and Soldaini: Archives Italiennes de Biologie, 1896, xxv, 465.

<sup>4</sup> Rudisch: Archiv f. Verdauungskrankheiten, 1909, xv, 469.

<sup>5</sup> Forscheimer: American Journal of Medical Sciences, 1911, cxli, 164.

<sup>6</sup> Mosenthal: Journal American Medical Association, 1913, lviii, 777.

<sup>7</sup> Macleod: American Journal of Physiology, 1908, xxii, 393.

<sup>8</sup> Eiger: Zentralblatt f. Physiologie, 1915, xxx, 445.

the glycogenic function, and what little effect they may have, if any, is entirely opposite to that reported by all except Mosen-thal and Macleod. We include a brief but complete report of the work quoted on this subject.

*Experiments of Morat. Dogs were used throughout. He did not record the weight*

Pilocarpine

ANIMAL	AMOUNT OF PILOCARPINE NITRATE	HOW GIVEN	INITIAL SUGAR	HIGHEST OR FINAL SUGAR	TIME AFTER ADMIN- ISTRATION
	grams				hrs.
1. Young dog.....	0.05	Subcutaneously	0.160	0.210	8-10
2. Young dog.....	0.05	Intraperitoneally	0.120	0.175	Some
3. Dog, in digestion	0.10	Intraperitoneally	0.075	0.190	8

Atropine Sulphate

				lowest sugar	
4. Young dog.....	0.05	Subcutaneously	0.145	0.119	15
5. Young dog.....	0.10	Subcutaneously	0.110	0.095	6
6. Young dog.....	0.15	Intraperitoneally	0.109	0.084	7

Cavazzani and Soldaini also worked with dogs. They administered chloral intraperitoneally. Then atropine was given in divided doses until the eyes dilated and the mouth was dry. The blood was taken from the *sushepatic*. The coeliac plexus was stimulated with a faradic current of such intensity that it could be tolerated by the hand. The stimulating electrode was large enough to stimulate all parts of the coeliac plexus. After about five minutes stimulation, blood was again taken for sugar analyses. From the results they conclude that atropine paralyzes the glycosecretory fibers. These conclusions are based on the following experiments:

WEIGHT OF DOG	AMOUNT OF CHLORAL	ATROPINE	HOW ADMINISTERED	INITIAL SUGAR	FINAL SUGAR	AFTER STIMULA- TION FOR
kg.	grams					min.
1. 6.5	2.60	0.015	Subcutaneously in divided doses	0.074	0.056	4
2. 3.5	1.40	0.015		0.114	0.107	5
3. 25.0	10.00	0.055		0.06	0.057	5
4. 5.0	2.00	0.03		Traces	None	

*Control animals without atropine. Except for lack of atropine, the technique in the controls was exactly as in the preceding*

DOG	INITIAL SUGAR	FINAL SUGAR
1. Weight not given.....	0.132	0.222
2. Weight not given.....	0.069	0.178
3. Weight not given.....	0.025	0.135
4. Weight not given.....	0.055	0.114
5. Weight not given.....	0.113	0.161
6. Weight not given.....	0.127	0.142

Eiger's work is based on the fact that the liver of the turtle is in two parts connected by bridges of tissue which he cut with a hot knife. The seared edges formed an impenetrable crust, giving practically two livers. An 0.05 to 0.15 per cent solution of dextrose was perfused through the two halves of the liver at a constant pressure while the vagus to one of the halves (right) was stimulated intermittingly. All of the visible branches of the vagus (left) to the other half of the liver were destroyed and the whole region brushed with concentrated phenol to destroy the connections between the two vagi. There was an increase of glycogen in the stimulated half of 10 to 14 per cent more than in the control half. Eiger reports only two experiments in which the actual figures quoted are not at all convincing. More experiments are needed before any conclusion should be drawn.

*His control experiment.* Perfusion fluid 700 cc. Ringer's solution plus 0.15 per cent dextrose in both lobes. Time of perfusion one hour and 25 minutes. At the end of this time the right lobe contained 5.60 per cent glycogen, and the left 5.54 per cent.

*Experiment 1.* Perfusion fluid, Ringer's solution plus 0.1 per cent dextrose. 800 cc. perfused through both lobes while the vagus to one lobe was stimulated. The time of the perfusion is not given. The stimulated lobe contained 5.826 per cent glycogen; the non-stimulated lobe contained 5.252 per cent glycogen.

*Experiment 2.* Perfusion fluid, Ringer's solution plus 0.05 per cent dextrose. 500 cc. perfused through each lobe. Duration of perfusion one hour and 33 minutes. The right vagus was stimulated during the perfusion. At the end of the perfusion the right lobe contained 5.51 per cent glycogen, the left lobe 4.83 per cent.



Macleod found that the free administration of atropine, 1 mgm. to 1.5 mgms. per kilo weight of animal, did not lessen the hyperglycemia produced by stimulation of the splanchnic nerve. My own results reported below are in entire accord with his and not at all in agreement with those of the other workers cited.

*The action of atropine on the normal animal*

I. Dog, 4 kilos

Normal sugar in the blood.....	0.058
Two hours after intravenous injection of 0.05 gram of atropine sulphate.....	0.100
Four hours after.....	0.060

II. Dog, 6 kilos

Normal sugar content.....	0.056
Fifteen minutes after the intravenous injection of 0.05 gram of atropine.....	0.072
Three hours after initial injection.....	0.075
Twenty-four hours afterward, animal excited by manipulation. . .	0.115

III. Old dog, weight 14 kilos. Fed two hours before on mixed food	
9.20 Sugar in blood.....	0.040
Given 0.05 atropine sulphate intravenously.	
9.35 Vomits.	
9.50 Blood sugar.....	0.078
12.40 Blood sugar.....	0.090
3.40 Blood sugar.....	0.068
9.20 Next day, blood sugar.....	0.040

IV. Young dog, 9 kilos

9.25 Blood sugar.....	0.051
Given 0.05 atropine sulphate intravenously.	
9.40 Vomits.	
9.55 Blood sugar.....	0.082
12.45 Blood sugar.....	0.046
3.45 Blood sugar.....	0.058
9.25 Next day, 24 hours after time of injection.....	0.045

These animals were somewhat excited for some hours after the injection. They whined and were in an uneasy mood, but otherwise no decided action beyond the usual atropine actions. The rise in the sugar content I believe, is due to the stimulating and exciting effect rather than to any specific action.

*Action of pilocarpine on the blood sugar content of normal animals*

## I. Dog, 5 kilos

Normal sugar.....	0.04
Ninety minutes after 0.004 gram pilocarpine nitrate.....	0.04

## II. Dog, 5 kilos

Normal sugar.....	0.04
Ninety minutes after 0.008 grams pilocarpine nitrate.....	0.05

## III. Dog 12 kilos, fed one hour before, on mixed diet

Normal sugar.....	0.051
Twenty minutes after 0.01 grams pilocarpine nitrate intravenously...	0.052

## IV. Dog, 15 kilos

Normal sugar.....	0.042
Four hours after 0.05 grams pilocarpine nitrate subcutaneously...	0.046
Seven hours after.....	0.032

## V. Dog, 10 kilos. Fed twelve hours before

Normal sugar.....	0.040
Four hours after 0.05 grams pilocarpine nitrate intraperitoneally.	0.020
Seven hours after.....	0.015
Twenty-four hours after.....	0.030

## VI. Dog, 10.5 kilos. Not fed since day before

Normal sugar.....	0.044
Four hours after 0.02 grams pilocarpine nitrate subcutaneously...	0.046
Seven hours after.....	0.066

## VII. Dog not in good health, 8 kilos

Sugar.....	0.038
Three hours after 0.03 grams pilocarpine nitrate subcutaneously..	0.020
Five hours afterward.....	0.026

The action of the drug in those cases that the sugar content fell, was extreme vomiting, diarrhoea, dribbling of urine, and great depression. I do not attribute the fall to any specific action but simply to fatigue. The noticeable feature is that there is no significant rise in the sugar content in any case.<sup>9</sup> If any action on the nerves governing secretion occurred, a rise should

<sup>9</sup> Cavazzani: Archives Italiennes de Biologie, 1896, xxv, 465.

be expected in a short time. The experiments definitely indicate that pilocarpine does not increase the blood sugar.

*The action of atropine on the rise in the blood sugar due to ether*

It is well known that anesthesia causes a rise of the blood sugar. Ross and McGuigan<sup>10</sup> found that the average rise in dogs on a meat diet after 30 minutes of ether anesthesia was 45 to 62 per cent, with those on a mixed diet, the rise was 56 per cent of the original amount. If, as is claimed, the formation of the sugar is modified by the administration of atropine or pilocarpine, then we should expect a significant change in the ether rise after the exhibition of these alkaloids. Such is not the case, however.

I. Dog, 10 kilos. Fed an hour before on mixed diet

No atropine, normal sugar.....	0.060
After fifteen minutes ether.....	0.096
Thirty minutes after removal of the ether.....	0.058
A. Three dogs weighing 15.5, 14.5, 10.5 kilograms were fed 250 grams of meat each thirty minutes before ether, and anesthetized for 15 minutes. The sugar rise was (1) 0.050 to 0.110 per cent; (2) 0.036 to 0.148 per cent; (3) 0.036 to 0.115 per cent	

II. Dog, 10 kilos. Fed on mixed diet one hour before

	<i>per cent</i>
Sugar 30 minutes after 0.05 grams atropine sulphate subcutaneously	0.051
After 15 minutes ether.....	0.080
Thirty minutes after removal of the ether.....	0.095

III. Dog, 15 kilos. Fed two hours before on a mixed diet

Normal sugar.....	0.066
Given 0.05 grams atropine subcutaneously and anesthetized five minutes later. Sugar after 15 minutes anesthesia.....	0.090
Thirty minutes after removal of the ether.....	0.105

IV. Dog, 15 kilos. Fed on mixed diet one hour before

Blood sugar 45 minutes after 0.05 grams atropine sulphate. ....	0.050
After 15 minutes ether.....	0.100
Thirty minutes after removal of the ether.....	0.124

<sup>10</sup> Ross and McGuigan: Journal of Biological Chemistry, 1915, xxii, 407.

## V. Dog, 13 kilos. Fed one hour before on mixed diet

Blood sugar 70 minutes after 0.03 grams atropine.....	0.055
After 15 minutes ether.....	0.096
Thirty minutes after the removal of the ether.....	0.120

## VI. Dog, 8 kilos. To test the action of atropine given after the administration of ether had commenced

Normal sugar.....	0.060
After 15 minutes ether.....	0.124
0.05 grams atropine sulphate now given subcutaneously and after 20 minutes more, sugar was.....	0.122

*The action of pilocarpine on the sugar rise due to ether*

## I. Dog, 10 kilos. Fed two hours before on a mixed diet

Normal sugar.....	0.033
Given 0.05 grams pilocarpine nitrate subcutaneously. After vigorous action of the pilocarpine (6 minutes) the animal was etherised. 15 minutes of ether raised the sugar to.....	0.123
Thirty minutes after removal of the ether, blood sugar.....	0.127
There was considerable disturbance of the respiration here. This is a terrific dose of pilocarpine.	

## II. Dog, 12 kilos. Fed about an hour before on a mixed diet

Normal sugar.....	0.051
Given 0.01 grams pilocarpine nitrate intravenously, and 30 minutes later, sugar.....	0.052
After 15 minutes ether.....	0.104
Thirty minutes after removal of the ether.....	0.062
Ether after pilocarpine therefore acts as on the normal animal.	

*Stimulation of the coeliac plexus*

## I. Dog, 7.5 kilos. Stimulation under ether alone, without atropine

9.00 Etherized.	
9.15 Blood sugar.....	0.080
9.23 Stimulated coeliac plexus-faradic 3 volts through primary coil with secondary removed 7.5 to 8 centimeters. This strength could be borne easily by the hand. The electrode was large enough to stimulate all parts of the plexus. This was the degree and method of stimulation used in each case. Stimulation continued for five minute periods with two minutes interruption for the time mentioned in each case.	
9.30 Sugar.....	0.150
10.00 Sugar.....	0.178

(Stimulus removed)



10.30 Sugar.....	0.150
Exposure of the intestines for one hour causes a drop of sugar to.....	0.132

## II. Dog, 14 kilos. Fed one hour before on mixed diet

9.50 Atropine 0.05 grams hypodermically.	
10.25 Sugar before ether.....	0.046
Thirty minutes after ether.....	0.080
Thirty minutes after coeliac stimulation.....	0.100
Thirty minutes after removal of stimulus.....	0.110
Atropine in this case did not stop the rise of the sugar by the ether or by the stimulation of the coeliac plexus.	

## III. Dog, 10 kilos

8.00 Given apomorphine which caused copious emesis.	
11.00 Atropine 0.02 grams subcutaneously.	
11.15 Blood sugar.....	0.052
11.15 Ether.	
11.30 Blood sugar.....	0.060
11.40 Stimulation of the coeliac plexus as above.	
12.05 Blood sugar.....	0.090
12.15 Blood sugar.....	0.100
12.45 Thirty minutes after removal of stimulus.....	0.124
1.15 Sixty minutes after removal of stimulus.....	0.159

## TECHNIQUE USED

The samples for sugar analyses were taken from the jugular vein with a hypodermic needle. About ten grams were used for each determination. The figures given are the averages of satisfactory duplicates. The proteins were removed by precipitation in a solution of 20 per cent sodium sulphate (Merck) containing 1.5 per cent acetic acid, heated to boiling. The blood was weighed in each case. The amount of sugar was determined by the Bertrand method.

The results may be summarized as follows:

1. Large doses of atropine usually cause an increase of the blood sugar, probably due to excitement.
2. Pilocarpine in large doses never causes a significant increase of the blood sugar, and often causes a reduction after some hours. The reduction is apparently due to depression and fatigue.

3. The increase in the blood sugar due to ether anesthesia is not modified by atropine or pilocarpine.

4. Atropine in massive doses, does not lessen the hyperglycemia due to stimulation of the coeliac plexus.

5. The influence of pilocarpine and atropine on the blood sugar furnishes no evidence of the presence of glycoscretory nerves.



## CROSS TOLERANCE

ALTERED SUSCEPTIBILITY TO CODEIN, HEROIN,  
CANNABIS-INDICA AND CHLORAL-HYDRATE IN  
DOGS HAVING AN ACQUIRED TOLERANCE FOR  
MORPHINE

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It is a well recognized clinical fact that individuals addicted to the use of alcohol, are more resistant to the narcotic effects of ether. This altered susceptibility is spoken of as a crossed tolerance. The manner in which it is produced is not clear. With habituation or tolerance to alcohol it is found that its rate of combustion in the body is increased considerably above the normal (1). This change in combustion rate however does not explain the tolerance to alcohol and in the absence of further knowledge the unsatisfactory expression, "lessened susceptibility of the nerve cells" is made use of. The tolerance to ether is explained in the same indefinite way, and presumably involves the same nerve cells. The pharmacological action of ether on the central system may be considered to be very similar to that of alcohol since both act on the same nerve cells in the same direction and neither has a selective action not shared in by the other. The pharmacological similarity together with the close chemical relationship would lead one to assume, even in the absence of known facts, that the toleration of alcohol and ether was mutual. Similarly a crossed tolerance between chloroform and the closely related chloral hydrate might be assumed. With other drugs however, which may be grouped together because of some ultimate general effect produced, as for example, sleep,



the assumption of a cross tolerance is not so well warranted nor are facts available to show whether such cross tolerance does occur. To quote from Sollmann (2)

It is an interesting fact that functional habituation, when acquired for a particular drug, may hold also for other drugs having a similar action. A habitual drunkard, for example, is resistant to the general anaesthetics. Whether this extended immunity also holds true of other allied drugs, such as morphin and cannabis indica, has not been determined.

The following work reports an attempt to determine whether such a crossed toleration does exist; first, between such closely related drugs as morphine and methyl and diacetyl morphine, and second, between morphine and other less related depressants of the central nervous system to which the cells become tolerant; e.g., cannabis indica and chloral hydrate. Cannabis indica represents drugs not chemically related but sharing in some of the selective actions of morphine, chloral hydrate such depressants as have neither chemical relationship nor selective actions in common.

It will be well to first consider the results of work done to determine the cause of toleration toward morphine; also the comparative pharmacologic effects of the drugs considered, through which we might expect crossed toleration to exist. Faust (3) found that a dog habituated to morphine excreted decreasing amounts of the drug though the dose were increased. This led to the natural conclusion that the decreased susceptibility was due to increased power of destruction. Cloetta (4) showed that the tissues of the central nervous system have an affinity for morphine, and in tolerant animals, acquire a marked ability to destroy it. That this acquired ability to destroy the poison is not the only factor concerned in producing a tolerance is shown by the fact that the amount of morphine in the general circulation of an animal with an acquired tolerance, during the height of absorption, is toxic to a corresponding normal animal (5). As in the explanation of alcohol toleration, a lessened susceptibility of the nerve cells is offered as an explanation.

It is interesting to note that tolerance to morphine is not acquired to an equal degree by the various nerve centers. For example, in dogs no longer narcotized by large doses of the drug, the vagus center continues to respond to comparatively small quantities (6).

In attempting to produce a tolerance for codein, Bouma (7) found neither decrease in the symptoms produced nor increase in the power of destruction. It is evident then, that in substituting one hydroxyl group of morphine with the methyl radicle, a marked change has been produced in the drug as regards its receptivity by the tissues. Such a change, it would seem, would be likely to modify the possibility of crossed toleration between morphine and codein more than the close chemical relationship would lead one to expect.<sup>1</sup> It does not, however, preclude the possibility of a crossed tolerance being established between the two. As mentioned before, the increased power of burning alcohol in the habitu  , is without doubt largely concerned in the establishment of tolerance for that drug. Notwithstanding the fact that ether and alcohol have a mutual toleration, the former is not oxidized in the body, but excreted unchanged.

Heroin is produced by substituting both hydroxyl groups of morphine with acetyl radicles. It is interesting to note, in contrast to codein, that in spite of the greater chemical change produced in making heroin from morphine, clinical reports of habituation and tolerance to heroin are not uncommon. No explanation of this tolerance has been given.

Chloral unites with glucuronic acid in the body, the resulting combination being inactive. Were the formation of the inactive glucuronate to keep pace with the increasing amounts of chloral taken, a clear explanation of chloral tolerance would be afforded. This however is not the case, nor as a matter of fact does an acquired chloral tolerance exist to any striking degree (8).

I have found no reference to a study of cannabis indica tolerance.

<sup>1</sup> A case of tolerance to codein is reported in the *Deut. Med. Woch.*, 1905, vol. 22, p. 864; stating that the condition was analogous to that occurring in morphine habituation.

In comparing the pharmacological actions of these drugs, it is especially noticeable that codein and heroin have but little of the analgesic effect of morphine. Large doses of the first two produce a strychnine-like stimulation of the cord. Morphine produces greater digestive disturbance and constipation than heroin or codein. Habituation occurs more readily with morphine than with either of the derivatives. While morphine may cause a mild diaphoresis, heroin has been advocated in checking the night sweats of phthisis. Even the paths of major excretion differ, codein being excreted mainly by the kidneys, morphine by the alimentary tract. These drugs, however, in common depress the respiratory center, and, in the dog, produce disturbance in equilibrium. It is in these respects we might expect their mutual toleration to be most evident.

*Cannabis indica* has been reputed to vary considerably in strength and keeping properties and also in regard to its action in man. In the dog its action is quite uniform, producing a depression of the central nervous system especially evidenced by slow, deep respirations, partial analgesia and disturbed control of equilibrium (9). This depression is occasionally preceded by mild stimulation and always followed by sleep lasting six to eight hours. Tolerance is rapidly acquired. It may be briefly stated that the action of *cannabis indica* in the dog resembles that of morphine more than it does the action of any other drug, not chemically related.<sup>2</sup>

Chloral hydrate depresses the central nervous system after the manner of chloroform and alcohol, sharing none of the selective actions of morphine.

#### METHOD

Toleration to morphine was produced as rapidly as possible, known solutions of the sulphate salt being injected subcutaneously, daily. The plan of the work has been to test the

<sup>2</sup> The preparation of *cannabis indica* used in this work was a fluid extract, "physiologically assayed," obtained fresh from the manufacturers. After two years storage at room temperature, exposed to sunlight, it had not varied in strength to an extent that would have been appreciable in dosage of 0.03 cc. per kilogram body weight.



comparative effects of the several drugs upon the same tolerant animals. In order to keep them in as good physical condition as possible, toleration was not increased above 10 mgm. per kilogram, except in one dog. The latter animal was made tolerant to 30 mgm. morphine per kilogram to make certain no error was escaping unnoticed in the animals having a lesser toleration. In normal dogs 1 mgm. per kilogram produces definite symptoms. The injections were given about three hours after the daily feeding, minimizing nutritional disturbance. It has been stated (3) that dogs habituated to morphine exhibit a desire for the customary injection of the drug. Quite the opposite was found to be the case with the dogs used in this study. Clean, sharp, small calibrated needles were introduced as painlessly as possible; the animals were treated with kindness, yet each showed a dread of the injection that could hardly be explained as being due to the introduction of the needle.

Codein and heroin were injected subcutaneously, cannabis indica and chloral hydrate given by stomach tube. In order to insure accurate dosage, before withdrawing the tube after giving cannabis indica, 2 cc. of 80 per cent alcohol followed by 2 cc. of 50 per cent alcohol were allowed to flow down the tube. After removing the tube, washing in alcohol gave but a slight green color. Chloral hydrate was given in 1 per cent solution, the tube being washed before removal with 10 cc. tap water.

Minimal effective doses were sought as best illustrating any degree of toleration. In order to minimize distraction of the dogs and permit the exhibition of the slightest symptoms, they were observed singly in a quiet room, without apparatus.

Test doses of drugs were given the habituated dogs approximately twenty-four hours after the last injection of morphine.

The above is a fair example of the difference in response between a normal dog and one tolerant to morphine when given a minimal effective dose (0.012 gram per kilogram) of codein phosphate. Comparing the condition of the normal dog twenty-six minutes after injection with that of the tolerant animal thirty-four minutes after receiving the drug, the most striking contrasts are the preliminary stimulation of the respiratory center and



TABLE 1  
*Effect of codein*

NORMAL					MORPHINE TOLERANT			
Time	Respiration	Heart	Remarks		Time	Respiration	Heart	Remarks
	23 Rhythmical Regular Shallow	100 Rhythmical with res- pirations Regular	Normal			14½ Rhythmical Regular Deep	76 Rhythmical with res- pirations Regular	Normal
0			0.012 gm. codein per kilogram	0				0.012 gm. co- dein per kilo- gram
14			Slight unstead- iness	14				No effects
17	32 Rhythmical Regular Moderately deep	128 Rhythmical with res- pirations regular	Defecation	34	13 Rhythmical Regular Deep	72 As above		
26	96 Panting type of respira- tion		Marked unstead- iness	64	12½ As above	76 As above		
46	24 Rhythmical Regular Shallow	96	Defecation	79	12½ As above	82 As above		No unstead- iness
52	18 Rhythmical Regular Shallow			99	13 As above	82 As above		No evident effect

TABLE 2  
*Effect of codein*

NORMAL				MORPHINE TOLERANT			
Time	Respiration	Heart	Remarks	Time	Respiration	Heart	Remarks
	21 Rhythmical Regular Shallow	104 Rhythmical with res- piration	Normal		12 Moderately deep Regular	128 Regular	Normal
0			0.012 gm. codein per kilogram	0			0.012 gm. co- dein per kilo- gram
30	24 As above	116 As above	Moderate un- steadiness	28	11 As above	104 No change except in rate	
37	Panting type of respira- tion Irregular Not counted			43	No change	No change	No unstead- iness
94	15 Deep Rhythmical Regular	96 Rhythmical with res- piration	Marked unstead- iness	58	11 Moderately deep Regular	104 Regular	As above
139			Unsteadiness di- minished	73	No change	No change	No change

marked unsteadiness in equilibrium of the former compared with lack of effect in the latter. An evident stimulation of peristalsis is also shown in the dog unaccustomed to morphine, no evidence of this action being shown by the habituated animal.

The same lack of response to codein in animals habituated to morphine is here shown, the minimal effective dose for a normal dog being used. The slightly decreased rate of respiration and heart beat in the tolerant dogs might well be explained by their confinement with a leash in a quiet room during the time under observation.

With an increase of 0.003 gram codein-phosphate per kilogram above the minimal effective dose, respiratory stimulation and following depression is more marked, there is a greater disturbance of equilibrium and more severe diarrhoea in a normal dog. The only evidence of action in a dog tolerant to morphine, with this dose, is a slightly unsettled equilibrium of short duration.

Forty milligrams of codein phosphate per kilogram in a dog unaccustomed to the action of morphine produces marked stimulation preceding a deep depression of the respiratory center. The cells governing equilibrium are also depressed. The cord shows evidence of excessive irritability.

In striking contrast a dog habituated to the action of morphine shows no signs of drug action upon the respiratory center, after such a dose of codein. The disturbance of equilibrium is of a mild degree. An unmistakable concern or fear is shown. It is interesting to note that the heart rate is increased in the morphine tolerant animal, occurring synchronously with the evidences of anxiety. This increased cardiac rate is produced in a morphine tolerant dog given a markedly toxic dose of codein (see table 8). One other point of interest is the lessened duration of action of codein, judging by the symptoms produced, in dogs habituated to morphine.

The minimal effective dose of heroin hydrochloride in a normal dog is 0.0001 gram per kilogram. With this dose no effect is noticed except a change in stability that is well described as a weakness in muscular control. Whining and anxiety is occa-

TABLE 3

*Effect of codein*

NORMAL					MORPHINE TOLERANT			
Time	Respiration.	Heart	Remarks		Time	Respiration.	Heart	Remarks
	22 Rhythmical Regular	128 Rhythmical Regular	Normal			16 Rhythmical Regular Deep	108 Rhythmical with respiration	Normal
0			0.015 gm. codein per kilogram		0			0.015 gm. codein per kilogram
8			Slight unsteadiness		20			No evidence of effect
10	Whining Preventing Counting	104 Rhythmical Regular			36	14½ As above	64 Rhythmical with respiration Regular	Slight unsteadiness
15	212 Panting type		Moderate unsteadiness		43			Moderate unsteadiness
20	Panting as above	84 Rhythmical Regular			50	12 Rhythmical Regular Moderately deep	64 As above	No increase in unsteadiness
25			Marked instability		85	12 As above	68 As above	Slight unsteadiness
30	22 Rhythmical Regular		Diarrhoea		100	13 As above	60 As above	No unsteadiness
100	12 Rhythmical Regular Deep	84 Rhythmical with respiration Regular	Slight unsteadiness		190	13 No change	72 Rhythmical with respiration Regular	



TABLE 4  
*Effect of codein*

NORMAL				MORPHINE TOLERANT			
Time	Respiration	Heart	Remarks	Time	Respiration	Heart	Remarks
	25 Rhythmical Regular Moderately deep	112 Rhythmical Regular	Normal		7½ Rhythmical Regular Deep	92 Rhythmical Regular with res- pirations	Normal
0			0.040 gm. codein per kilogram	0			0.040 gm. co- dein per kilo- gram
4	24 As above	104 Rhythmical Regular		9	8 Rhythmical Regular Deep	96 Rhythmical Regular with res- piration	No evidence of effect
6	Whining Preventing Counting		Anxiety shown Mild instability	13	8 As above	144 Rhythmical Regular	Anxiety shown No instability
10	Panting type Too rapid to count		Marked instabil- ity	21	7½ As above	148 As above	As above
14	Panting Rate in- creased	96 Rhythmical Regular	Unable to stand	31	7½ No change	150	Mild instabil- ity
20	Panting al- ternating with rap- id respi- rations		Twitching of fa- cial muscles	38	8	146	As above

24	96	Arhythmi- cal Irregular Shallow	84	As above		53	8	142	
33	68	As above			Spasmodic con- tractions of muscle groups	83	8	116	Regular Rhythmical with res- piration
39	48	Rhythmical Shallow	86		Spasmodic con- tractions of ex- tremities				
49	26	Rhythmical Regular Shallow							
54	22½	As above	92	Rhythmical Regular					
59	18½	Rhythmical Regular							
66	15	Rhythmical Regular Moderately deep	92						
79	11	As above	88	Rhythmical Regular	Prostrated Excessive reflex irritability				

TABLE 5  
*Effect of heroin*

NORMAL					MORPHINE TOLERANT			
Time	Respiration	Heart	Remarks		Time	Respiration	Heart	Remarks
	16 Regular Rhythmical Shallow	88 Regular Rhythmical	Normal		20	Regular Rhythmical Moderately deep	64* Regular Rhythmical	Normal
0			0.0001 gm. heroin in per kilogram		0			0.0001 gm. heroin per kilogram
12	16 Regular Rhythmical Moderately deep	60 Regular Rhythmical	No evidence of effect		7	19½ Regular Rhythmical Moderately deep	64 As above	No effect
27	15 As above	60 As above	Muscular weakness		25	20 As above	64 No change	No evidence of effect
42	15 No change	60 No change	Too depressed to stand		65	19½ No change	68 No change	As above
82	No change	No change	As above					

TABLE 6  
*Effect of heroin*

NORMAL					MORPHINE TOLERANT			
Time	Respiration	Heart	Remarks		Time	Respiration	Heart	Remarks
	15 Regular Rhythmical Moderately deep	76 Regular Rhythmical	Normal			10 Regular Rhythmical Deep	69 Rhythmical with respiration	Normal
0			0.0001 gm. heroin in per kilogram		0			0.0001 gm. heroin per kilogram
.6			Whining Uneasiness		13			No evidence of effect
8	13½ Regular Rhythmical	68 Regular Rhythmical	Muscular weakness		23	11 Regular Rhythmical Deep	62 No change	No weakness No defecation
20	13 As above	72	Defecation Weakness more marked		33	11½ No change		
50	13 No change	80 Regular Rhythmical			45			No change
65	13 No change	80 No change						



sionally shown. A dog tolerant to morphine exhibits no symptoms with this dosage.

Heroin hydrochloride when given to a normal dog in dosage of 0.0002 gram per kilogram produces marked respiratory stimulation of short duration, followed by long continued depression. With this dose the animal is unable to stand during the height of the effect. A dog habituated to morphine shows no symptoms with this dose of heroin. The minimal effective dose of heroin in a dog tolerant to morphine is 0.0003 mgm. per kilogram; producing no effect upon the respiratory center, no defecation and but slight depression of muscular control.

The preceding chart shows the effects of large doses of codein and heroin in dogs tolerant to morphine. Codein phosphate 100 mgm. per kilogram produced such extreme irritability of the cord that a convulsive paroxysm could be produced by clapping, tapping the floor or lightly touching the animal. Although the respirations were arrhythmical, irregular and shallow during the height of the convulsive action, there was no change that could be interpreted as a stimulation or depression of the respiratory center. The recovery of the animal from this dose was rapid.

Heroin was injected, 0.003 mgm. per kilogram. This is thirty times the minimal effective dose in a normal dog. Although the dog was unable to stand after receiving this dose, there was no respiratory change that could be interpreted as due to the drug.

The above protocol serves to show the lack of toleration for cannabis indica shown by the dogs habituated to morphine.

Chloral hydrate given over a wide range of doses shows no evidence of altered susceptibility, in dogs tolerant to morphine.

#### SUMMARY

1. A marked crossed tolerance exists to codein and to heroin in dogs habituated to morphine, in so far as effects upon the respiratory center are concerned.

2. A slight crossed tolerance exists between codein and morphine, and between heroin and morphine in regard to their actions upon the tissues governing equilibrium.

TABLE 7  
*Effect of heroin*

NORMAL				MORPHINE TOLERANT			
Time	Respiration	Heart	Remarks	Time	Respiration	Heart	Remarks
	24 Rhythmical Regular Moderately deep	124 Rhythmical Regular	Normal		14½ Rhythmical Regular Moderately deep	86 Rhythmical with res- piration regular	Normal
0			0.0002 gm. her- oin per kilo- gram	0			0.0003 gm. her- oin per kilo- gram
17			Slight weakness	16	13½ As above	84 As above	Slight weak- ness
20	26 As above	120 As above	Marked weak- ness	19	13 As above	86 As above	
22	180 Panting type	120 No change	Unable to stand	23	15 Change in rate only		
26	244 Panting type	120 No change		47	16	72	
28	32 Intermit- tent with panting		Unable to stand	53	17	74	
36	25	112		57	14½		
66	14	100		67	12½ Rhythmical Regular Deep	70 Rhythmical with res- piration	
96	13 Rhythmical Regular Deep	90 Rhythmical with res- pirations		98	13	74	No further change
				113	13½	76	As above

TABLE 8  
*Effect of large doses of codein and heroin in dogs tolerant to morphine*

CODEIN				HEROIN			
Time	Respiration	Heart	Remarks	Time	Respiration	Heart	Remarks
	20 Rhythmical Regular Moderately deep	144 Rhythmical Regular	Normal		22 Rhythmical Regular Moderately deep	110 Rhythmical Regular	Normal
0			0.100 gm. codein per kilogram	0			0.003 gm. heroin per kilogram
8			Defecation	5	22 As above	108 As above	Slight general depression Mild weakness
10	20 As above	144 As above	Moderate un- steadiness	7			Moderate weakness
16	20 No change	140 No change	General depres- sion Marked un- steadiness	8			Marked weakness Falls
21			Defecation Highly excitable	11	24 Rhythmical Regular Moderately deep	88 Rhythmical with res- piration regular	Unable to stand

36	19	Arhythmical Irregular Shallow	.	Head retracted	19	24	As above	64	As above	
41	19	Less arhythmia	180	Arhythmical Convulsive movements	25	23	No change	68	No change	Higher centers apparently not depressed
46	17				33	21½	As above	72	As above	
56	16	Rhythmical Moderately deep	176	Arhythmical Convulsive movements Marked depression	45	20	As above	70	As above	
66	16	As above	146	Rhythmical Regular Diminishing convulsive movements	60	20	No change	72	As above	
76	17	As above	140	As above	85	20	No change	78	No change except in rate	
86	18	No change	136	As above Appearance quite normal						



TABLE 9  
*Effect of cannabis indica*

TIME	NORMAL			TIME	MORPHINE TOLERANT		
	Respiration	Heart	Remarks		Respiration	Heart	Remarks
	22 Rhythmical Regular Shallow	120 Rhythmical Regular	Normal		26 Rhythmical Regular Shallow	168 Rhythmical with res- piration regular	Normal
0			0.150 cc. canna- bis indica per kilogram	0			0.150 cc. can- nabis indica per kilogram
7	26 No change except in rate	142 No change except in rate	No evidence of drug effect	7	29 No change except in rate	164 As above	No evidence of effect
16	30 Rhythmical Regular Moderately deep	144 As above		17	30 As above	160 As above	As above
18			Slight unstead- iness	30	26 Rhythmical Regular Moderately deep	160 No change	Slight unstead- iness
30	24	140 Rhythmical with res- piration regular	Moderate un- steadiness	40	25 As above	152 No change except in rate	As above

55	22 Rhythmical Regular Moderately deep	132 As above	Moderate un- steadiness Drowsy	50	21 No change except in rate	152 As above	Moderate un- steadiness
70	22 No change	132 No change	As above	61	20 No change	156 As above	Drowsy
115	23 No change	102 Rhythmical with res- piration regular	Slight unsteadiness	71	19 Rhythmical Regular Moderately deep	152 No change	Moderate un- steadiness
				84	20 No change	144 Rhythmical with res- piration regular	As above

TABLE 10  
*Effect of chloral hydrate*

NORMAL				MORPHINE TOLERANT			
TIME	Respiration	Heart	Remarks	TIME	Respiration	Heart	Remarks
	22 Rhythmical Regular Moderately deep	116 Rhythmical Regular	Normal		18 Rhythmical Regular deep	100 Rhythmical Regular	Normal
0			0.080 gm. chloral hydrate per kilogram	0			0.080 gm. chloral hy- drate per kilogram
22	20 As above	108 Rhythmical Regular	No evidence of effect	30	16 As above	96 As above	Slight unstead- iness
34	18 No change except in rate	102 No change except in rate	Slight unstead- iness	45	16 No change	92 No change	Drowsy Lies down
40	18 As above	96 As above	Increased un- steadiness	65	16 No change	92 No change	Depression as above
50	18 No change	96 No change	Drowsy Lies down	75	16 No change	92 No change	
87	17 Rhythmical Regular Not quite as deep	94 Rhythmical with res- piration	As above	85	16 No change	92 No change	No change
110	17 As above	94 As above	No change				

3. Dogs tolerant to morphine when given codein or heroin have increased intestinal peristalsis.

4. No evidence of crossed toleration to cannabis indica or to chloral hydrate exists in dogs tolerant to large amounts of morphine.

5. The experiments cited show that a cross tolerance may exist between closely related drugs but that this tolerance is evidenced only on those functions in which the drugs have a common selective action.

#### CONCLUSION

As far as this study shows, therefore, a crossed tolerance involves only those structures upon which the drugs exert effects mutually alike.

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# THE ABSORPTION OF POTASSIUM IODID BY THE THYROID GLAND IN VIVO, FOLLOWING ITS INTRAVENOUS INJECTION IN CONSTANT AMOUNTS

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In an earlier paper by one of the authors in collaboration with Dr. Feiss,<sup>1</sup> it was shown that artificially perfused and surviving thyroids of dogs take up KI very rapidly and retain it in large amounts; that this activity is not shared by other tissues of the body; that KCN inhibits this activity and that only surviving thyroid cells manifest this phenomenon. At that time two experiments were reported in which 50 mgm. KI were injected intravenously, after having removed a control lobe of the thyroid. The lobes exposed to the KI for one hour showed practically the same affinity for this salt as was found in the in vitro perfusions.

In the present communication we will record the results obtained from a series of 33 experiments in which the KI was introduced intravenously. The plan of these experiments was as follows: In all but four experiments, dogs with grossly enlarged thyroids were used. After ligating the renal vessels of both kidneys and removing one lobe of the thyroid as a control, 50 mgm. KI in and 1 cc. distilled water was injected into the internal jugular vein, or one of its branches, below the thyroid area. Ether for anesthesia was the only drug used and in each case the usual aseptic technique was followed. The animals were allowed to live for periods of 5 minutes, 10 minutes, 1 hour, 4 hours, 8 hours, 12 hours, 16 hours, 20 hours, 24 hours and 30 hours following the injection of KI. In four experiments—two of 5 minutes duration, and two of 10 minutes duration—the renal vessels were not ligated. Then the iodized lobes were removed,

<sup>1</sup> The absorption of potassium iodid by perfused thyroid glands and some of the factors modifying it. *J. Pharmacol. and Exp. Therap.*, 1915, vii, 557.

TABLE 1

EXPERIMENT NUMBER	DURATION OF EXPERIMENT	WEIGHT DOG	WEIGHT OF CONTROL LOBE		IODIN CONTROL PER GRAM	TOTAL I CONTROL	WEIGHT OF IODIZED LOBE		IODIN PER GRAM IODIZED	TOTAL I IODIZED	IODIN INCREASE PER GRAM DRIED	THYROID WEIGHT PER KILOGRAM OF BODY WEIGHT	HISTOLOGY	I PER GRAM DRIED- SPLEEN	I PER GRAM DRIED- LIVER
			Fresh	Dried			Fresh	Dried							
					gram	gram			gram	gram	gram	gram			
330(A298)	5 minutes	5.00	10.5	2.10	0.00	0.00	13.0	2.6	0.71	1.84	0.71	0.38	Mod-Hyperpl.	trace	trace
32(A325)	5 minutes	9.20	4.0	0.87	2.36	2.05	3.0	0.8	2.61	2.06	0.25	3.06	Colloid	0.062	0.03
33(A326)	5 minutes	5.70	1.0	0.19	1.23	0.23	0.8	0.2	1.63	0.25	0.40	7.12	Normal	0.040	0.03
330(A323)	10 minutes	6.2	3.5	0.74	0.79	0.58	4.8	0.9	1.38	1.28	0.59	1.30	Early Hyperpl.	0.06	0.02
31(A324)	10 minutes	10.4	3.5	0.61	1.50	0.92	3.3	0.6	2.42	1.35	0.92	3.18	Normal	0.02	0.02
1(A249)	1 hour		25.5	5.10	0.32	1.63	29.0	5.8	0.77	4.47	0.45		Mod-Hyperpl.	0.06	0.03
2(A291)	1 hour		31.0	6.20	0.12	0.74	25.0	5.0	0.48	2.40	0.36		Mkd-Hyperpl.	0.06	0.03
3(A296)	4 hours	11.1	32.0	6.20	trace		69.5	13.9	0.95	13.20	0.95	0.15	Mod-Hyperpl.	0.00	0.00
4(A295)	4 hours	7.0	4.0	0.80	0.86	0.69	4.0	0.8	1.20	0.96	0.34	1.74	Colloid-Goiter	0.00	0.02
27(A320)	4 hours	10.5	29.0	5.74	0.00		25.0	4.4	0.80	3.49	0.80	0.41	Probable cancer areas	0.00	0.00
5(A297)	8 hours	13.0	66.0	13.20	0.18	2.38	49.5	9.9	0.62	6.14	0.44	0.26	Early-Hyperpl.	0.00	0.00
6(A299)	8 hours	10.2	62.5	17.30	0.38	6.57	52.0	12.6	0.57	7.18	0.19	0.19	Early-Hyperpl.	0.00	0.00
25(A318)	8 hours	6.0	28.0	6.92	3.38	23.37	21.0	5.6	4.00	22.47	0.62	0.28	Colloid	0.00	0.00
26(A319)	8 hours	14.5	11.5	2.51	0.15	0.38	12.5	2.7	1.11	3.01	0.96	1.15	Mod-Hyperpl. (un- comp)	0.00	0.00
7(A300)	12 hours	8.8	5.5	1.10	trace		6.5	1.3	0.28	0.36	0.28	1.34	Mkd-Hyperpl.	0.02	0.00
8(A301)	12 hours	6.4	9.5	1.90	trace		5.0	1.0	0.93	0.93	0.93	1.27	Mod-Mkd-Hyperpl.	0.00	0.00
23(A316)	12 hours	4.7	18.0	3.53	0.09	0.32	18.0	3.8	1.23	4.67	1.14	0.26	Mod-Mkd-Hyperpl.	0.00	0.00
24(A317)	12 hours	5.4	66.5	15.09	0.15	2.26	58.0	9.4	0.62	5.85	0.47	0.09	Comp-Colloid Mod	0.00	0.00
9(A302)	16 hours	12.4	10.0	2.00	1.11	2.22	9.0	1.4	1.54	2.16	0.43	1.37	Colloid	0.00	0.00

10(A303)	16 hours	10.4	10.0	2.00	0.49	0.98	9.5	1.9	0.71	1.35	0.22	1.09	Colloid	0.00	0.00
21(A314)	16 hours	9.2	4.0	0.66	0.15	0.99	4.0	0.7	0.62	0.44	0.47	2.30	Mkd-Hyperpl.	0.00	0.00
22(A315)	16 hours	12.0	31.0	6.85	0.46	3.15	26.5	6.8	1.11	7.55	0.65	0.45	Colloid	0.00	0.00
29(A322)	16 hours	11.8	29.0	5.83	0.18	1.05	23.0	5.1	0.92	4.73	0.74	0.51	Colloid-early	0.00	0.00
11(A304)	20 hours	10.8	6.5	1.80	2.28	4.10	7.0	2.0	2.52	3.07	0.24	1.54	Colloid	0.00	0.00
12(A307)	20 hours	15.0	15.0	3.08	0.12	0.37	14.5	3.4	0.77	2.61	0.65	1.03	Mod-Hyperpl.	0.00	0.00
20(A313)	20 hours	7.2	8.5	1.09	0.62	0.67	9.5	1.4	1.11	1.57	0.49	0.75	Colloid	0.00	0.00
28(A321)	20 hours	28.6	17.5	4.19	0.68	2.85	21.5	5.6	0.68	3.84		1.33	Colloid-early	0.00	0.00
13(A305)	24 hours	6.1	10.5	2.42	0.36	0.87	15.0	3.4	0.74	2.49	0.38	0.40	Colloid	0.00	0.00
14(A306)	24 hours	8.8	13.0	2.35	0.31	0.73	19.5	3.8	1.04	3.93	0.73	0.45	Mod-Hyperpl.	0.00	0.00
17(A310)	24 hours	7.5	10.5	2.38	1.69	4.01	15.5	4.1	2.21	9.06	0.52	0.48	Colloid	0.00	0.00
18(A311)	24 hours	12.9	7.0	1.23	0.00		7.0	1.3	0.46	0.59	0.46	1.84	Mkd-Hyperpl.	0.00	0.00
15(A308)	30 hours	8.6	100.0	17.1	0.00		71.0	12.8	0.92	7.91	0.62	0.12	Mkd-Hyperpl. (com- plie)	0.00	0.00
16(A309)	30 hours	6.6	21.0	3.8	0.02	0.06	15.5	2.9	1.85	5.27	1.70	0.42	Colloid-Mod	0.00	0.00



weighed and small sections taken for histology, and the remainder of the thyroid, together with pieces of liver and spleen desiccated for iodine determinations. As shown in the complete tabulation (table 1) the thyroid lobes used varied markedly in size, in iodine content and physiologic activity, as indicated by the range of histological appearances from quiescent or colloid, to marked active hyperplasia.

As shown in the *in vitro* perfusions the amount of KI absorbed necessarily varies with the surface exposed (size of glands) and the stage of physiological activity (colloid or normal glands showing the least increase in iodine). Any analysis of the quantities of iodine absorbed from a given dose must take into consideration both the size and the stage of physiologic activity of the glands used.

In all experiments there was a great increase in the iodine content of the thyroid lobe thus exposed to KI just as was observed in the *in vitro* perfusions. There are variations in the amounts retained by the thyroid from the constant amount (50 mgm.) offered, and some of the factors which might be related to these variations have been analyzed as follows:

1. Relation of the duration of the perfusion to the amount of KI retained. The principal data bearing on this point have been grouped in the following table, both as to duration of the perfusion and the histologic condition of the glands used—whether active hyperplasias or quiescent glands:

DURATION OF EXPERIMENT	HYPERPLASIAS		COLLOID GLANDS	
	Number of experiments	Average increase in I per gram dried in milligram	Number of experiments	Average increase in I per gram dried in milligram
5 minutes	1	0.71	2	0.32
10 minutes	2	0.75		
1 hour	3	0.53		
4 hours	2	0.87	1	0.34
8 hours	3	0.53	1	0.62
12 hours	4	0.70		
16 hours	2	0.60	3	0.43
20 hours	2	0.57	1	0.24
24 hours	2	0.59	2	0.45
30 hours	2	1.16		

The most striking features brought out in this tabulation are, (1) that the absorption of KI is so rapid during the first few minutes that the slight further increase during the rest of the experiment is masked, and (2), that it varies directly with the degree of active hyperplasia present or inversely with the original iodine content, and therefore is identical in all essentials with the results obtained in the *in vitro* perfusions. Concerning the first point, viz., the rapidity of absorption, it was a surprise to us to find so small a difference between a 5-minute or a 10-minute and a 30-hour perfusion, although the rapidity of the storage of iodine by the gland has been many times and from many different angles emphasized.

This series of experiments indicate that the absorption from the blood is practically instantaneous. In the earlier experiments the kidneys were removed from the circulation partly to bring this series of experiments into relation with the *in vitro* perfusions, and partly to eliminate any loss of KI through the kidneys. This precaution was found to have been unnecessary. In the four experiments where the renal vessels were not ligated no appreciable difference was observed in the percentage of iodine absorbed. The affinity of the thyroid for iodine salts is so great that the loss through the kidney is negligible when iodine is administered in physiological doses for thyroid effects. Even when given in large doses (a decigram of KI) it is doubtful whether the renal factor modifies the amount retained by the thyroid. The thyroid has such an extraordinary affinity for iodine and the other tissues have such a slight affinity for it, that the intravenous injection of iodine salts in the living animal may truly be designated as "*in vivo*" perfusion of the thyroid.

2. In sharp contrast with the thyroid, the liver and spleen show no retention of KI. Samples of liver and spleen were examined for iodine in each experiment, and only in the experiments of 1 hour or less duration was it detected. Traces of iodine were detected in the unwashed tissues of all such experiments just as it was found in the unwashed spleens and kidneys of the *in vitro* perfusions, but even traces could not be detected

when the tissue was thoroughly freed of blood. In all the experiments of 4 hours or longer, no detectable amounts of iodine were found in the liver and spleen.

#### SUMMARY

There is apparently no difference between *in vitro* and "*in vivo*" perfusions as regards the percentage of iodine absorbed. The absorption is practically instantaneous in each case. Maximum thyroid effects are produced by such exceedingly small amounts of iodine and the gland has such an extraordinary affinity for salts of iodine, that its loss through the kidney may be considered negligible, and this probably holds true for all other body tissues. The size of the gland and the stage of physiological activity modify the amount of KI absorbed apparently to the same degree whether it is introduced by *in vitro* perfusion or injected intravenously in the living animal.

The liver and spleen show no retention of KI, whether introduced by *in vitro* perfusion or by intravenous injection. With constant amounts of KI introduced and with glands of similar degrees of physiologic activity, there is no noteworthy difference in the percentage absorbed, whether the "*in vivo*" perfusion lasts 1 hour or 30 hours. There must be some slight increase in the amount of iodine absorbed from a single dose in the succeeding minutes or hours of a given experiment, but it was not sufficiently marked to be detected as an increase in the iodine content of the thyroid, in this series of glands with the methods employed; although after an hour it was not present in detectable amounts in the circulation.



## SOME NEW TIME RECORDING APPARATUS

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In securing a time record on pharmacological tracings, as in blood pressure experiments, simplicity and positive, rather than negative, indications of the time interval are considerations of some importance. Simplicity is easily secured, with the apparatus now available, if one is willing to make a record of one time interval serve all purposes. Thus in most experiments it is desirable to know the time of making an injection (or other experimental procedure), the duration of the injection, the time of onset of physiological changes resulting therefrom and their duration, and the time between various injections. This multiplicity of factors which are to be observed with reference to their time relations, can be determined from a record of a single time interval, if a second interval is used. But it will be at a considerable expenditure of time and effort. Also a considerable time interval cannot be determined with such unfailing accuracy by counting seconds as it could be by a simultaneous record of second and longer intervals.

On account of these factors some investigators are willing to sacrifice some simplicity so as to secure simultaneous records of different time intervals, such as, for example, second, ten second and minute intervals. The chief difficulty with this more complex time record is, that with every increase in the number of intervals recorded it is necessary to increase the primary unit equipment of time clock and signal magnet by an additional unit. In one laboratory a weight driven, a spring driven and an electrical driven clock, together with three signal magnets, were used to secure the three desired time records. In this laboratory, until recently, two electrically driven clocks



were used which, by certain modifications, gave three time intervals with two recording magnets.

With such a complex system difficulties were constantly arising, although the factor causing the greatest annoyance was the difficulty of so regulating the various clocks that they would remain exactly together. Frequently their relation, although the same at the beginning, would be entirely changed at the end of an experiment. Furthermore the space taken up by the additional signal magnets and time interval tracings was neither desirable nor was it free from complexity in certain cases where space was an item.

In spite of these undesirable factors three simultaneous time records have been in use in this laboratory and attempts have been made to simplify the arrangements for securing them. At the present time the three time records consisting of second, ten second, and minute intervals are secured from one clock, to interrupt the magnet current, and from one signal magnet.

#### THE ELECTRIC CLOCK

The electrical clock (Harvard Apparatus Company) seemed to lend itself most easily to certain modifications whereby different magnets could be activated by electrical contacts through the one clock. In this clock each swing of the pendulum turns a wheel, made up of several segments containing contact points arranged in parallel rows, past an adjustable contact. The contact, giving passage to the electrical current activating the magnet, is made every 1, 5, 15, 30, or 60 seconds depending on which row of contact points on the wheel is engaged by the adjustable contact. The pendulum turns this toothed wheel by means of a drag travelling in the seconds row.

When second intervals are being recorded by the more recent models of the electrical clock, three separate adjustments travel in the seconds row, the pendulum drag, the contact making the circuit to the magnet which swings the pendulum, and the adjustable contact making the current to the signal magnet. The contact to the signal magnet is momentary but if this con-

tact could be prolonged once in every minute a plateau would be obtained on the record which could easily be distinguished from the up and down stroke, without plateau, of the seconds contacts. The space between two of the contact points in the seconds row of the wheel could be blocked, providing a continuous current to the signal magnet over a space of one second, were it not for its also blocking the two other adjustments in this row. For these reasons an additional seconds row was built into the wheel and, after blocking the space between two of the contacts, the adjustable contact was moved along the rigid bar supporting it into the new seconds row, thus providing contacts giving second and minute intervals from one signal magnet.

To secure ten second intervals a second adjustable contact was placed on the rigid supporting bar, and after proper insulation from the original adjustable contact, it was connected with its own pair of binding posts through which the current, made at the moment of contact with the toothed wheel, was carried to a second signal magnet. This adjustable contact was moved into a row on the toothed wheel provided with six contact points, thus completing the circuit through the signal magnet every ten seconds.

#### THE SIGNAL MAGNET

With the modification of the electrical clock, giving three simultaneous time intervals, two signal magnets were required to record these intervals. It then became desirable to eliminate one of these magnets and it was seen that one writing armature could be made to record the three time intervals if a magnet could be devised which would permit this armature to write either up or down from a common base line, or position of arrest.

Fundamentally the development of such a magnet is a very simple matter for, by merely combining two separate magnets on a single base, one armature will move either up or down, whether the magnets are on opposite sides of the armature or on the same side but on opposite sides of its fulcrum. The practical difficulty with such an arrangement is in securing the

arrest of the writing armature as its spring pulls it away from the magnet after the electrical circuit is broken. This difficulty was obviated by the use of an armature for each magnet, one to

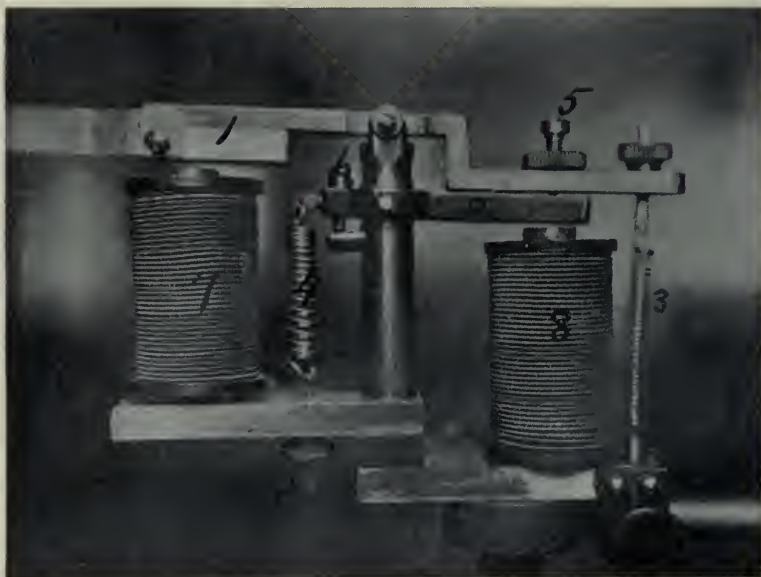


FIG. 1. SIGNAL MAGNET

1, Armature carrying writing point; 2, armature serving as arrest for 1; 3, spring to bring armature 1 to rest; 4, spring to bring armature 2 to rest; 5, adjustment screw to control the fling of the writing point down; 6, adjustment screw to control the fling of the writing point up; 7 and 8, magnets receiving electrical currents from different sources; 9, fine adjustment to swing writing point to or away.

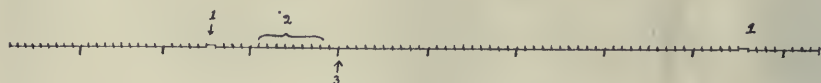


FIG. 2. TRACING OF TIME RECORD

1, Minute record; 2, seconds record; 3, ten seconds record.

carry a writing point and the other to serve as an arrest for the writing armature. Two magnets were placed on a common base separated by a post which served as the fulcrum for both armatures. The upper armature, carrying the writing point at

one end, was extended on the other side of the fulcrum so as to rest on the armature of the second magnet which thus served to arrest its movement when pulled into place by its spring. The armature of the second magnet is arrested by a fixed block as in the ordinary magnet. The circuit through the magnets comes to one, from the seconds and minute adjustable contact on the clock, to the other, from the ten seconds adjustable contact, so that the movement is upward for the second and minute intervals and downward for the ten second intervals.

The reproduction of a photograph of this magnet will make clear the details of its arrangement. The binding posts for the attachment of the two pair of wires from the clock are on the opposite side and do not show in the photograph.





## ON THE PERIPHERAL ACTION OF THE OPIUM ALKALOIDS. EFFECT ON THE SENSORY NERVE TERMINALS

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Although a number of opium preparations for local use are still included in the United States, British, and other pharmacopeias and in the National Formulary, and although various opiates have been and still are extensively used by clinicians locally in the form of lotions, ointments, plasters, suppositories and the like, the general opinion among the majority of modern pharmacologists is that opium is devoid of any local action on sensory nerve terminals. Some go even so far as to ridicule the empirical employment of opium by practitioners in this manner and to characterize such practice as "pharmacological superstition." (1)

If we turn from mere belief or disbelief and speculation, to scientific inquiry and experimental evidence on the subject, we find surprisingly few data upon which to base a rational and unbiased opinion. Indeed the few experimental data at our disposal do not at all disparage the local use of the drug.

It is well known, for instance, that dionin or ethyl morphin is a powerful local anesthetic and has been used as such in ophthalmic surgery (2); the same is true of benzoyl morphin or peronin. Moukhtar (3), in an interesting experimental work on guinea pigs, found that intradermal injections of minute quantities of solutions of opium alkaloids show a distinct local effect on the sensory nerve terminals, which effect is not due to mechanical distension of tissue, as he proved by control injections of physiological saline solution. Even more interesting is the accidental discovery by Pal (4) of the locally anesthetic action of papaverin. On tasting a little papaverin sulphate he noted a numbness of

the tongue. Further investigation by him showed that the alkaloid possesses marked local anesthetic properties, so that on applying it to a rabbit's eye, a painless operation could be performed.

In a previous issue of this journal, one of the present authors (M.) in conjunction with Messrs. N. B. Herman and C. S. Levy reported a quantitative study of the analgesia produced by the central action of opium alkaloids individually and in combination with each other, after injection and absorption in normal men (5).

It was but a logical step to apply the same method to an investigation of the topical action of opium alkaloids.

#### METHOD

The method has been described in the paper just mentioned and depends on the production of finely graded pain stimuli by means of a large induction coil. The normal sensory thresholds for various spots on the surface of the body were first determined, and were found to be remarkably constant for any one spot. A drug was then administered by subcutaneous or intramuscular injection, and after its absorption the sensory threshold was again observed. The result according to whether the threshold was higher or lower than normal indicated an analgesic or hyper-algesic effect of the drug.

As in the preceding research, being fully aware that certain subjective elements, inherent in the character of the investigation, entered into our experiments, we have taken painstaking precautions to eliminate all errors arising from this source. To this end numerous control experiments were performed.

Each experiment was carried out in the same room, under perfectly constant conditions. Readings were always taken with the subject in the same position; perfect quiet was observed; and even draughts were eliminated as possible disturbing factors. The subject was not allowed to look at the apparatus, but always sat with eyes closed or averted and fixed on some other object.

The electrodes employed were of course the same throughout the experiments. The ends were blunt so as to avoid mechanical stimulation. The distance between the electrodes was kept

fixed. Care was taken to maintain a constant wetness of the surfaces stimulated. The pressure with which the electrodes were applied was kept approximately constant, and even the direction of the application was the same. The observer or experimenter manipulating the coil, took care to move the secondary at a constant rate of speed.

When a drug was applied, the subject was ignorant of its nature. Furthermore, as controls, normal saline, distilled water, and solutions of various inactive substances were often substituted in place of the drug, without the subject's knowledge. It may be added that owing to the paucity of data on the subject and owing to the conflicting experiences of the few previous observers, we were ignorant of the pharmacological effect to be expected from the alkaloids studied, thus further eliminating any subjective bias.

Previously to studying the effect of the opium alkaloids of whose local action we were entirely ignorant, tests were made on eight subjects, with our best known local anesthetic cocain, in order to determine whether the method described above was applicable at all to a study of local anesthesia or analgesia. For this purpose, solutions of 2 per cent and 3 per cent of cocain hydrochloride were applied locally to various surfaces of the body and the pain threshold determined before and after application. The spots generally studied were on the tongue, the lips, and the back of the hand between the thumb and forefinger. In case of the hand the skin previously to the experiment was thoroughly scrubbed with soap and water to render it soft and more permeable.

The results of the cocain experiments were very striking and may be illustrated by the following protocol:

*Experiment, December, 1915. H. J. B. Drug, cocain hydrochloride 2 per cent*

REGION	NORMAL THRESHOLD C. G. S. UNITS	DURATION OF APPLICATION	THRESHOLD AFTER DRUG C. G. S. UNITS
		<i>minutes</i>	
Hand.....	2277	3	4209
Lip.....	1278	2	2139
Tongue.....	1725	1	7590



It will be noted that not only were the lip and tongue anesthetized, as could be felt by the numbness in those places, even without testing with the electric current, but that in case of the skin of the hand a distinct rise in the pain threshold was observed. This called for some comment. It has been generally held by the older pharmacologists that watery solutions of cocain do not penetrate the unbroken skin. From our experiments with cocain, alypin, and other local anesthetics, there seems to be no doubt that the pain threshold is raised by the local application of these drugs. That this was not due to a difference in conduction, produced by differences in concentration of the solutions used was shown by control experiments. Wetting the skin with plain water, with physiological (0.8 per cent) salt solution, with 4 per cent NaCl solution, and with Locke's solution, with the electrodes firmly applied to the surface, produced no appreciable change in the threshold. We must, therefore, assume that the solutions do penetrate far enough to affect the nerve terminals, especially after the skin has been scrubbed with soap and water; or that in the course of the experiment minute breaks in the epidermis may be produced by the electrical stimulation and otherwise, at the points of the application of electrodes, and in that way the drug gets to the nerve pain terminals. Cathaphoresis can hardly be regarded as playing any part in the experiments, as we are dealing not with a galvanic current, but with an alternating, interrupted current, and although the break stimulation is a trifle greater than the make shock, the positive and negative electrodes were interchanged in the various experiments without any appreciable effect. On referring to authoritative works on anatomy, and on personally consulting a number of anatomists and physiologists, the authors have learned that sensory nerve terminals extend much closer to the surface of the skin than is generally supposed, and that it is quite plausible that the solutions of the drugs tested may reach the nerve terminals even without assuming a break in the skin. Thus Kölliker (6) describes free nerve endings in the epidermis. In the histology of Böhm, Davidoff and Huber (7), a remarkable figure illustrating such endings is shown, and one anatomist, in a

personal communication went even so far as to state that in his opinion nerve endings may reach the surface and be shed with the rest of the skin. On the other hand it has been shown by microscopical examination of sections of skin, that even very weak aqueous solutions of picric acid, of silver nitrate, and various dyes applied to the surface of the skin, soon penetrate into the deeper layers of the epidermis. Again it is well known that immersion of the hand for a few minutes in a very weak solution of phenol will result in a distinct tingling of the skin.

It is to be remembered, however, that the action of cocain just described is strictly on the nerve *endings*, that is, quite superficial, so that it is not an anesthesia in the surgical sense of the word.

Having determined the applicability of the method in case of cocain, and other well-known local analgesics, a systematic inquiry into the local action of opium alkaloids by the same method was thereupon begun. The observations were for the most part made on Dr. Macht and Messrs. Bollinger and Johnson, with occasional readings on other subjects. The spots studied were generally in three different regions of the body, namely, on the skin of the back of the hand; on the semi-moist mucous membrane of the lips, and on the moist mucous membrane of the tongue. Keeping the surfaces examined (hand and lip) uniformly moist with a physiological solution of sodium chloride, the normal sensory threshold was determined. A solution of the alkaloid or other substance to be tested was then applied with a pledget of cotton to the spot, and after a brief interval of time the sensory threshold was again determined.

The time of application of the drug was purposely made as short as possible. It was generally three minutes for the hand, two minutes for the lips, and one minute for the tongue. Occasionally the drug was allowed to remain for longer periods of time. The skin was always scrubbed with soap and water before an experiment. Many control experiments were made without the subject's cognizance. The results were extremely interesting and are indicated in the tables below. All the readings were expressed in terms of the position of the secondary coil, and in terms of C. G. S. units, as described in the previous work on analgesia.

## ACTION OF INDIVIDUAL ALKALOIDS

The most striking effects were noticed after local application of papaverin. We were not only able to confirm qualitatively Pal's observations with the concentrated solutions of the alkaloid (10 per cent) which he used, but also with much weaker

TABLE 1  
*Effect of Papaverin*

SUBJECT	REGION	STRENGTH OF DRUG	DURATION OF APPLICATION	NORMAL THRESHOLD		EFFECT OF DRUG	
				Readings in centimeters	C. G. S.	Readings in centimeters	C. G. S.
			<i>min.</i>				
D. I. M.	{ Hand	Papaverin hcl. 4%	3	9.7	6210	9.0	7590
	{ Lip	Papaverin hcl. 4%	2	11.8	2829	10.9	4002
	{ Tongue	Papaverin hcl. 4%	1	10.8	4209	9.8	5934
H. J. B.	{ Hand	Papaverin hcl. 1%	3	12.4	2277	12.0	2622
	{ Lip	Papaverin hcl. 1%	2	15.0	1019	14.4	1173
	{ Tongue	Papaverin hcl. 1%	1	13.5	1588	12.9	4897
H. J. B.	{ Hand	Papaverin hcl. 4%	3	14.1	1312	13.1	1794
	{ Tongue	Papaverin hcl. 4%	1	13.5	1588	12.6	2139
S. L. J.	{ Hand	Papaverin hcl. 4%	3	10.9	4002	10.5	4692
	{ Lip	Papaverin hcl. 4%	2	14.2	1278	13.2	1725
	{ Tongue	Papaverin hcl. 4%	1	13.8	1449	11.2	3588
S. L. J.	{ Hand	Papaverin hcl. 1%	3	11.3	3450	11.0	3864
	{ Lip	Papaverin hcl. 1%	1	13.7	1520	13.2	1725
	{ Tongue	Papaverin hcl. 1%	1	13.4	1622	12.6	2139
H. J. B.	{ Hand	NaCl 0.9%	3	11.6	3036	11.6	3036
	{ Lip	NaCl 0.9%	2	12.5	2208	12.5	2208
	{ Tongue	NaCl 0.9%	1	14.8	1070	14.8	1070
Dr. R.	Tongue	Papaverin hcl. 5%	3	10.3	5106	7.6	10902
Dr. L.	Lip	Papaverin hcl. 5%	2	10.6	4554	9.2	7314
D. I. M.	Tongue	Papaverin hcl. 4%	1	10.5	4692	8.0	9936
Dr. B.	Lip	Papaverin hcl. 4%	2	10.7	4347	9.2	7314
Dr. M.	Lip	Papaverin hcl. 4%	2	10.8	4209	10.1	5451
Dr. L.	Lip	Papaverin hcl. 4%	2	9.8	5934	8.8	8004
C. C.	Lip	Papaverin hcl. 5%	2	10.6	4554	8.2	9384
	{ Hand	Papaverin hcl. 4%	3	12.0	2622	11.7	2967
S. L. J.	{ Lip	Papaverin hcl. 4%	2	13.3	1656	12.8	1966
	{ Tongue	Papaverin hcl. 4%	1	14.7	1104	14.0	1346
	{ Hand	Papaverin hcl. 4%	3	14.1	1312	13.1	1794
H. J. B.	{ Hand	Papaverin hcl. 4%	2	15.5	915	14.5	1156
	{ Tongue	Papaverin hcl. 4%	1	13.5	1588	12.6	2139



TABLE 2  
*Effect of Morphin*

SUBJECT	REGION	STRENGTH OF DRUG	DURA- TION OF APPLI- CATION	NORMAL THRESHOLD		EFFECT OF DRUG	
				Read- ings in centi- meters	C. G. S.	Read- ings in centi- meters	C. G. S.
			min.				
D. I. M.	{ Hand	Morphin sul. 4%	3	11.0	3864	10.2	5313
	{ Lip	Morphin sul. 4%	2	13.1	1794	12.8	1966
	{ Tongue	Morphin sul. 4%	1	13.0	1863	11.0	3864
D. I. M.	{ Hand	Morphin sul. 2%	3	9.5	6624	9.0	7590
	{ Lip	Morphin sul. 2%	2	13.2	1725	12.2	2484
	{ Tongue	Morphin sul. 2%	1	12.5	2208	11.0	3864
H. J. B.	{ Hand	Morphin sul. 4%	3	11.3	3450	11.0	3864
	{ Lip	Morphin sul. 4%	2	15.0	1019	14.0	1346
	{ Tongue	Morphin sul. 4%	1	13.5	1588	12.8	1966
H. J. B.	{ Hand	Morphin sul. 2%	3	10.0	5520	9.2	7314
	{ Lip	Morphin sul. 2%	2	14.0	1346	13.0	1863
	{ Tongue	Morphin sul. 2%	1	12.9	1897	11.9	2760
H. J. B.	{ Hand	Morphin sul. 3%	3	14.0	1346	13.8	1449
	{ Lip	Morphin sul. 3%	2	17.5	552	17.3	588
	{ Tongue	Morphin sul. 3%	1	12.5	2208	12.5	2208
H. J. B.	{ Hand	Morphin sul. 2%	3	11.7	2967	11.1	3626
	{ Lip	Morphin sul. 2%	2	13.7	1520	13.4	1622
	{ Tongue	Morphin sul. 2%	1	11.5	3174	11.2	3588
H. J. B.	{ Hand	Morphin sul. 3%	3	12.3	2346	11.0	3864
	{ Lip	Morphin sul. 3%	2	14.2	1278	12.8	1966
	{ Tongue	Morphin sul. 3%	1	12.0	2622	10.5	4692
S. L. J.	{ Hand	Locke's sol. (control)	3	10.6	4554	10.6	4554
	{ Hand	Morphin sul. 4%	3	10.6	4554	9.6	6384
	{ Lip	Locke's sol. (control)	2	13.3	1656	13.3	1656
S. L. J.	{ Lip	Morphin sul. 4%	2	13.3	1656	12.2	2484
	{ Tongue	Locke's sol. (control)	1	12.7	2070	12.8	1966
	{ Tongue	Morphin sul. 4%	1	12.7	2070	12.0	2622
S. L. J.	{ Hand	NaCl. (control)	3	9.4	6831	9.3	7038
	{ Hand	Morphin sul. 2%	3	9.3	7038	8.8	8004
	{ Lip	NaCl (control)	2	12.6	2139	12.6	2139
S. L. J.	{ Lip	Morphin sul. 2%	2	12.6	2139	11.7	2967
	{ Tongue	NaCl (control)	1	12.8	1966	12.8	1966
	{ Tongue	Morphin sul. 2%	1	12.8	1966	11.6	3036
S. L. J.	{ Hand	Morphin sul. 3%	3	10.8	4209	9.4	6831
	{ Lip	Morphin sul. 3%	2	12.1	2553	11.4	3312
	{ Tongue	Morphin sul. 3%	1	12.1	2553	10.9	4002
S. L. J.	{ Hand	Morphin sul. 2%	3	10.5	4692	9.6	6384
	{ Lip	Morphin sul. 2%	2	13.6	1554	12.0	2622
	{ Tongue	Morphin sul. 2%	1	12.1	2553	9.1	7452
S. L. J.	{ Hand	Morphin sul. 2%	3				
	{ Lip	Morphin sul. 2%	2	12.0	2622	11.4	3312
	{ Tongue	Morphin sul. 2%	1	11.0	3864	10.5	4692
S. L. J.	{ Hand	Morphin sul. 4%	3	9.5	6624	9.4	6831
	{ Lip	Morphin sul. 4%	2	12.0	2622	11.5	3174
	{ Tongue	Morphin sul. 4%	1	11.8	2829	10.6	4554



solutions. A solution of 4 per cent of papaverin hydrochloride caused distinct and marked analgesia, and solutions of 2 per cent and even 1 per cent of the salt gave distinctly measurable results. The effect, as might be expected, was most marked on the tongue, and the numb sensation experienced was very much like that produced by local application of cocain. Table 1 illustrates the action of papaverin.

The effect of morphin was greater than was expected. Solutions of 4 per cent produced an analgesia distinctly measurable. Solutions of 2 per cent occasionally produced an appreciable lowering of the sensory threshold as measured by the method employed, but in other cases did not. A solution of 1 per cent was not effectual. The results of morphin experiments are shown in table 2.

In regard to the other opium alkaloids it was found that codein produces but a slight local effect. The phosphate, sulphate, and hydrochloride were employed, in 5 per cent solution. Narcotin, which is closely allied to papaverin, also produced a distinct local analgesia almost as marked as after papaverin, but experiments with it were not conveniently made because of the total insolubility of the alkaloid itself, and the acid reaction of the solutions of its salts. In this case controls had to be made with other acidulated solutions. Narcein produced a distinct change in the sensory threshold. Thebain produced only very little change. Table 3 illustrates the results obtained with the alkaloids.

#### EFFECT OF COMBINED OPIUM ALKALOIDS

Having tested the local action of the individual opium alkaloids, it was interesting to inquire into the effect of opium itself or of the combination of the various alkaloids. This was especially desirable, because in the previous work on analgesia, a distinctly synergistic effect was noted. For this purpose experiments were made with local applications of Sahli's combination of total opium alkaloids, which is sold under the name of pantopium or pantopon. It was found that dilutions of pantopon as low as 1 per cent were sufficient to produce a dis-

TABLE 3  
*Effect of Codein, Narcotin, Narcein, and Thebain*

SUBJECT	REGION	STRENGTH OF DRUG	DURATION OF APPLI- CATION	NORMAL THRESHOLD		EFFECT OF DRUG	
				Read- ings in centi- meters	C. G. S.	Read- ings in centi- meters	C. G. S.
			<i>min.</i>				
D. I. M.	{ Hand	Codein phosph. 5%	3	12.0	2622	11.6	3036
	{ Lip	Codein phosph. 5%	2	17.2	605	15.7	863
	{ Tongue	Codein phosph. 5%	1	14.0	1346	13.0	1863
A. R.	{ Hand	Codein phosph. 5%	3	11.0	3864	10.8	4209
	{ Hand	Codein phosph. 5%	3	11.2	3588	11.2	3588
H. J. B.	{ Lip	Codein phosph. 5%	2	16.4	708	16.4	708
	{ Tongue	Codein phosph. 5%	1	14.2	1278	14.2	1278
H. J. B.	{ Hand	Codein phosph. 5%	6	11.2	3626	11.2	3626
	{ Lip	Codein phosph. 5%	4	16.4	708	15.8	829
	{ Tongue	Codein phosph. 5%	1	14.2	1278	13.7	1520
S. L. J.	{ Hand	NaCl 0.9%	3	11.5	3174	11.5	3174
	{ Lip	NaCl 0.9%	2	15.5	915	15.5	915
	{ Tongue	NaCl 0.9%	1	14.5	1156	14.5	1156
S. L. J.	{ Hand	Codein hcl. 5%	3	11.5	3174	11.2	3588
	{ Lip	Codein hcl. 5%	2	15.5	915	15.0	1019
	{ Tongue	Codein hcl. 5%	1	14.5	1156	13.8	1449
H. J. B.	{ Hand	Narcotin hcl. 0.5%	3	12.1	2553	11.8	2829
	{ Lip	Narcotin hcl. 0.5%	2	15.0	1019	14.5	1156
	{ Tongue	Narcotin hcl. 0.5%	1	13.3	1656	13.0	1863
S. L. J.	{ Hand	Narcotin hcl. 0.5%	3	10.5	4692	10.0	5520
	{ Lip	Narcotin hcl. 0.5%	2	13.7	1520	12.8	1966
	{ Tongue	Narcotin hcl. 0.5%	1	12.8	1966	12.4	2277
D. I. M.	{ Hand	Narcein 2.5%	3	14.1	1312	12.6	2139
	{ Lip	Narcein 2.5%	2	16.2	742	15.2	966
	{ Tongue	Narcein 2.5%	1	14.1	1312	13.1	1794
D. R.	{ Hand	Narcein 2.5%	3	12.5	2208	12.0	2622
	{ Lip	Narcein 2.5%	2	14.2	1278	13.8	1449
	{ Tongue	Narcein 2.5%	1	13.3	1656	14.0?	1346?
H. J. B.	{ Hand	Narcein 2.5%	3	12.0	2622	11.1	3626
	{ Lip	Narcein 2.5%	2	17.1	614	16.2	742
	{ Tongue	Narcein 2.5%	1	16.8	639	15.6	897
S. L. J.	{ Hand	Narcein 2.5%	2	12.2	2484	11.8	2829
	{ Lip	Narcein 2.5%	2	15.8	829	15.2	966
	{ Tongue	Narcein 2.5%	1	15.4	932	14.4	1173
D. I. M.	{ Hand	Thebain hcl. 5%	3	12.0	2622	11.7	2967
	{ Lip	Thebain hcl. 5%	2	13.7	1518	13.3	1656
	{ Tongue	Thebain hcl. 5%	1	13.4	1622	11.7	2967

TABLE 3—Continued

SUBJECT	REGION	STRENGTH OF DRUG	DURATION OF APPLI- CATION	NORMAL THRESHOLD		EFFECT OF DRUG	
				Read- ings in centi- meters	C. G. S.	Read- ings in centi- meters	C. G. S.
			<i>min.</i>				
H. J. B.	{ Hand	Thebain hcl. 5%	3	11.5	3174	11.3	3450
	{ Lip	Thebain hcl. 5%	2	15.0	1019	13.3	1656
	{ Tongue	Thebain hcl. 5%	1	13.9	1380	12.6	2139
S. L. J.	{ Hand	Thebain hcl. 5%	3	11.3	3450	11.0	3864
	{ Lip	Thebain hcl. 5%	2	13.5	1588	12.5	2208
	{ Tongue	Thebain hcl. 5%	1	13.8	1449	13.1	1794
D. I. M.	{ Hand	Narcotin hcl. 0.5%	3	11.8	2829	10.9	4002
	{ Lip	Narcotin hcl. 0.5%	2	17.5	552	16.0	778
	{ Tongue	Narcotin hcl. 0.5%	1	15.3	949	13.2	1725
S. L. J.	{ Hand	Narcotin hcl. 0.5%	3	11.2	3588	10.7	4347
	{ Lip	Narcotin hcl. 0.5%	2	15.4	932	14.4	1173
	{ Tongue	Narcotin hcl. 0.5%	1	15.0	1019	13.9	1380

tinect change in the sensory threshold. The composition of pantopon according to Sahli (8) is 50 per cent of morphin and 40 per cent of all the other alkaloids, of which the principal one is narcotin. Inasmuch as in our experiments with individual

TABLE 4  
*Effect of Pantopon*

SUBJECT	REGION	STRENGTH OF DRUG	DURATION OF APPLI- CATION	NORMAL THRESHOLD		EFFECT OF DRUG	
				Read- ings in centi- meters	C. G. S.	Read- ings in centi- meters	C. G. S.
			<i>min.</i>				
D. I. M.	{ Hand	Pantopon 1%	3	10.9	4002	9.0	7590
	{ Hand	Pantopon 1%	5	10.9	4002	8.0	9936
D. I. M.	{ Hand	Pantopon 1%	5	13.5	1588	12.5	2208
	{ Lip	Pantopon 1%	3	15.0	1019	14.4	1173
	{ Tongue	Pantopon 1%	2	12.6	2139	12.0	2622
D. I. M.	{ Hand	Pantopon 2%	3	13.5	1588	12.2	2484
	{ Lip	Pantopon 2%	2	15.0	1019	13.2	1794
	{ Tongue	Pantopon 2%	1	12.6	2139	11.8	2829
D. I. M.	{ Hand	Pantopon 2%	3	13.4	2277	12.0	2622
	{ Lip	Pantopon 2%	2	15.1	1002	13.2	1725
	{ Tongue	Pantopon 2%	1	14.5	1156	12.9	4897

TABLE 4—Continued

SUBJECT	REGION	STRENGTH OF DRUG	DURATION OF APPLI- CATION	NORMAL THRESHOLD		EFFECT OF DRUG	
				Read- ings in centi- meters	C. G. S.	Read- ings in centi- meters	C. G. S.
			<i>min.</i>				
D. I. M.	{ Hand	Pantopon 1%	3	10.5	4692	10.4	4899
	{ Lip	Pantopon 1%	2	12.1	2553	11.8	2829
A. R.	{ Finger	Pantopon 2%	35	12.9	3312	11.4	4897
	{ Hand	Pantopon 1%	5	14.0	1346	13.6	1554
S. L. J.	{ Lip	Pantopon 1%	3	15.3	948	14.8	1070
	{ Tongue	Pantopon 1%	1	15.7	863	15.0	1019
	{ Hand	Pantopon 2%	3	10.0	3864	10.4	4899
S. L. J.	{ Lip	Pantopon 2%	2	13.0	1863	12.6	2139
	{ Tongue	Pantopon 2%	1	13.0	1863	12.6	2139
S. L. J.	{ Finger	NaCl 0.9%	15	10.5	4692	10.5	4692
	{ Finger	Pantopon 1%	15	10.5	4692	9.4	6831
	{ Hand	Pantopon 1%	3	11.9	2760	11.6	3036
S. L. J.	{ Lip	Pantopon 1%	2	13.2	1725	12.7	2070
	{ Tongue	Pantopon 1%	1	13.2	1725	12.8	1966
S. L. J.	{ Finger	Pantopon 2%	15	14.0	1346	13.2	1725
	{ Hand	Strych. sulph. 0.01%	3	10.5	4692	10.5	4692
S. L. J.	{ Lip	Strych. sulph. 0.01%	2	12.2	2884	12.2	2884
	{ Tongue	Strych. sulph. 0.01%	1	12.2	2884	12.2	2884
	{ Hand	Pantopon 4%	3	12.5	2208	12.1	2553
S. L. J.	{ Lip	Pantopon 4%	2	15.7	863	15.1	1002
	{ Tongue	Pantopon 4%	1	15.2	966	14.4	1173
	{ Hand	Pantopon 1%	3	11.2	3588	10.9	4002
S. L. J.	{ Lip	Pantopon 1%	2	14.3	1244	13.6	1554
	{ Tongue	Pantopon 1%	1	14.2	1278	13.2	1725
	{ Hand	Pantopon 2%	3	14.2	1278	13.9	1380
H. J. B.	{ Lip	Pantopon 2%	2	17.7	518	16.8	639
	{ Tongue	Pantopon 2%	1	16.6	673	16.1	759
	{ Hand	Pantopon 1%	3	13.3	1656	13.1	1794
H. J. B.	{ Lip	Pantopon 1%	2	16.0	778	15.8	829
	{ Tongue	Pantopon 1%	1	14.9	1036	14.4	1173
	{ Hand	Strych. sulph. 0.01%	3	13.1	1794	13.1	1794
H. J. B.	{ Lip	Strych. sulph. 0.01%	2	15.3	949	15.6	847
	{ Tongue	Strych. sulph. 0.01%	1	13.6	1554	14.0	1346
	{ Hand	Pantopon 4%	3	15.3	948	14.2	1278
H. J. B.	{ Lip	Pantopon 4%	2				
	{ Tongue	Pantopon 4%	1	16.7	656	15.7	863
	{ Hand	Pantopon 1%	3	12.5	2208	12.0	2622
H. J. B.	{ Lip	Pantopon 1%	2	14.1	1312	14.1	1312
	{ Tongue	Pantopon 1%	1	13.6	1554	13.0	1863
S. L. J.	{ Finger	Pantopon 2%	15	14.0		13.3	



alkaloids, the lowest effective dose of morphin required to reduce the local sensibility was 2 per cent and that of narcotin and papaverin not less than 0.5 per cent, it follows that the local analgesia produced by the mixture is not due to an arithmetical summation of the actions of the individual alkaloids, and is another example of synergism.

#### DISCUSSION

From the above experiments it is seen that the various opium alkaloids, when applied locally to the skin or mucous membranes, exert a distinct effect upon the sensory nerve endings, raising the sensory threshold as indicated by the greater amount of electrical stimulation required to produce the first sensation of pain. The most efficient in this respect is papaverin; next in order is narcotin; third comes morphin; with narcein, codein, and thebain following in the order of their efficiency.

It has been furthermore noted that a mixture of the total opium alkaloids (pantopon) exerts this effect even in a greater degree than the amount of morphin or papaverin which it contains would do, if applied alone. From this it follows that the different alkaloids seem to potentiate each other, just as they do, when injected subcutaneously or intravenously and absorbed by the blood.

Without presuming to magnify its importance, it is evident, therefore, that opium, although primarily a central nervous poison, does also act to some extent upon peripheral structures, i.e., sensory nerve endings, and that the empirical observations of the older clinicians, who advocated local applications of opiates for the relief of pain, are not totally without foundation.

This would seem to be even more plausible, if we bear in mind that in the present research, the time of application was purposely made as short as possible (generally one to five minutes) on the one hand, while on the other hand, the opium applications employed in clinical practice are allowed to remain in contact with the skin of mucous membranes over long periods of time.

## SUMMARY

1. A quantitative study of the pain threshold before and after local application of various opium alkaloids, shows that they exert a distinctly measurable action on the sensory nerve terminals, producing a slight analgesic effect.

2. In the order of their efficiency in this respect the opium alkaloids may be arranged as follows: Papaverin, narcotin, morphin, narcein, codein, and thebain.

3. A combination of total opium alkaloids was found to be more effective than the amount of morphin, or of papaverin or narcotin, it contains would be if given alone.

4. The local effect of opium observed in this investigation, agrees well with numerous empirical observations by clinical men in the past and present.

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## THE LETHAL DOSE OF ARSENIC FOR SPLENECTOMIZED MICE

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Since splenectomy is frequently performed as a treatment for severe anaemias and since the condition of the patient subsequently requires, in many cases, other therapy; either blood transfusion or the administration of arsenic; it seemed worth while to determine, by animal experimentation, whether the removal of the spleen influences the lethal dose of the poison.

The distribution of arsenic in the body, both of normal individuals and of persons whose death was due to arsenic poisoning, has occupied the attention of numerous physiological chemists. In the former (1) series the interest was rather qualitative than quantitative, because of the minute amounts involved; in the latter (2) the majority of the workers centered their attention upon the arsenic content of the liver, kidneys and nervous system. v. Zeynek, however, says that the spleen contains nearly as many milligrams of arsenic per gram of tissue as does the liver, which is generally agreed to be the chief storage place for the poison. Garnier (3) found the liver content to be 5 mgm. of arsenic per 100 grams of liver substance, in a patient who had died of acute arsenic poisoning; v. Zeynek, (4) under similar conditions, found 5.9 mgm. of arsenic per 100 grams of liver. The latter investigator adds that the splenic content of his case was 4.3 mgm. of arsenic per 100 grams of spleen, and that this was the same amount that he found in the kidney substance.

The total amount of the poison which is contained by the liver and the spleen varies widely because of the difference in their size in the normal individual. The weight of the spleen, how-



ever, in some cases of pernicious anaemia, approaches more nearly to that of the liver than does the normal organ.

In reference to the direct relation of a single organ to the lethal dose of arsenic, Jeandelize and Perrin (5) say that the thyroid plays an important role in death from this poison. These workers used rabbits and repeated doses of the sodium salt of arsenic were administered. They found that the thyroidectomized animals invariably died after fewer doses of the poison than the normal ones, also that the lethal dose for the animals that had been deprived of their thyroids was from  $33\frac{1}{3}$  to 5 per cent of the amount required to kill the normal control animals. If the administration of the drug was stopped at the time of the death of the thyroidectomized rabbits the normal rabbits lived. Whether this result was due to a generally less active metabolism and a consequent undue retention of the harmful substance within the body tissues; or whether it resulted from some more remote connection of the gland and its products with the poison was not determined; though the authors inclined to the latter interpretation.

Recent work on the infection of splenectomized mice with tuberculosis gives a particular interest to the relation of the spleen to poisons. It has been shown that a white mouse which has been splenectomized is more resistant to the *Bac. tuberculosis* than is a normal animal to inoculation with the same strain of organisms. This increased resistance is explained by Lewis and Margot (6), who reported it first, as due to a specific substance which is produced by the spleen and which favors the development of these bacilli within the human body. They call it tuberculosplenin.

Murphy and Ellis (7) found that the period of increased resistance of the splenectomized mice to tubercular infection began three weeks after operation and was coincident with an enlargement of the lymph nodes and a lymphocytosis in the circulating blood. These workers, therefore, felt that the protection enjoyed by splenectomized mice was due to the activity of those organs which hypertrophy after the removal of the spleen.

Wilberg (8) determined the lethal dose of arsenic for various animals. He used the commercial preparation of potassium arsenite, which is an unknown combination of potassium meta-arsenite and arsenious acid; he subjected the powder to a drying process which materially reduced its weight. Wilberg found that white mice were considerably more resistant to arsenic poisoning than other laboratory animals and that from 0.0156 mgm. to 0.0176 mgm. of the substance per gram mouse produced marked symptoms and that 0.016 mgm. to 0.018 mgm. had a lethal effect. Wilberg recognized, therefore, an overlapping of the symptomatic and the lethal dose, and gives a variation of 0.002 mgm. of poison per gram mouse in the lethal dose for normal animals.

The first series of mice that were used in my experiments received arsenious acid in 1 per cent solution neutralized with potassium bicarbonate; that is, Fowler's solution without the coloring and flavoring substance. This special preparation was diluted 1:100 with distilled water so that the animals received the neutralized arsenious acid in a dilution of 1:1000. In accordance with the findings of Hunt (9) that the reaction of the body tissues to poisons is influenced markedly by the diet, the animals used for this work were kept at least one week, and usually longer than ten days upon a fixed diet of bread and water before the arsenic was injected. The bread was always obtained from the hospital kitchen and was the same throughout the experiment.

Some preliminary injections were made with sodium cacodylate; but this substance had to be abandoned because of its lack of toxicity; 1 mgm. per gram mouse was used without observable result. After the lethal dose of the prepared Fowler's solution was determined the following series of six normal and five splenectomized mice were injected. No animal was injected a second time, the diet was carefully supervised and the mice lived in jars filled with hay, the injection was subcutaneous and usually made in the back.

*Injection of 1: 1000 solution of arsenious acid neutralized by potassium bicarbonate.  
Normal mice*

DATE OF DIET	DATE OF INJECTION	WEIGHT	ARSENIOUS ACID	MGMS. PER GRAM MOUSE	RESULT
		<i>grams</i>	<i>mgms.</i>		
Dec. 1	Dec. 21	16	0.12	0.0075	Died in 24 hours
Dec. 1	Dec. 21	11	0.08	0.0073	Died in 48 hours
Dec. 1	Dec. 21	10	0.075	0.0075	Died in 48 hours
Dec. 1	Dec. 21	12	0.09	0.0075	Died in 24 hours
Dec. 1	Dec. 21	14	0.1	0.0071	Survived
Dec. 1	Dec. 21	9	0.067	0.0074	Died in 24 hours

*Injection of 1: 1000 solution of arsenious acid neutralized by potassium bicarbonate  
(Splenectomized mice)*

DATE OF DIET	DATE OF OPERATION	DATE OF INJECTION	WEIGHT	MGMS. PER GRAM MOUSE	RESULT
			<i>grams</i>		
Dec. 1	Dec. 6	Dec. 22	18	0.0075	Survived
Dec. 1	Dec. 6	Dec. 22	12	0.0075	Survived
Dec. 14	Dec. 14	Dec. 22	10	0.0075	Survived
Dec. 13	Dec. 16	Dec. 22	12	0.0075	Survived
Dec. 13	Dec. 20	Dec. 22	14	0.0072	Killed by accident

Following this result<sup>1</sup> potassium arsenite was used for a second series of subcutaneous injections. The mice for this series were kept in cotton waste instead of in hay as being less likely to interfere with the constancy of the diet. Twelve normal and six splenectomized mice were used. The potassium arsenite was made up into solution each time 0.1 gram to 100 cc. of distilled water.

<sup>1</sup> Mallinckrodt Chemical Works, St. Louis.



# LETHAL DOSE OF ARSENIC FOR SPLENECTOMIZED MICE 469

## *Injection of normal mice with potassium arsenite (1:1000 dilution)*

DATE OF DIET	DATE OF INJECTION	WEIGHT	ARSENITE	MGMS. PER GRAM MOUSE	RESULT
		<i>grams</i>	<i>mgms.</i>		
Feb. 14	Mar. 25	10	0.13	0.013	Died in 48 hours
Feb. 14	Mar. 28	10	0.13	0.013	Survived
Feb. 14	Mar. 28	15	0.195	0.013	Survived
Feb. 14	Mar. 28	17	0.221	0.013	Survived
Feb. 14	Mar. 28	13	0.169	0.013	Survived
Apr. 11	Apr. 27	20.1	0.301	0.015	Died in 6 hours
Apr. 11	Apr. 27	21.5	0.322	0.015	Died in 6 hours
Apr. 11	Apr. 28	18	0.25	0.014	Died in 24 hours
Apr. 11	Apr. 28	20	0.28	0.014	Died in 7 days chronic poisoning
Apr. 11	May 9	19	0.266	0.014	Survived
May 16	May 24	14.5	0.2	0.014	Survived
May 16	May 24	15	0.21	0.014	Died in 24 hours

## *Injection of splenectomized mice with potassium arsenite (1:1000 dilution)*

DATE OF DIET	DATE OF OPERATION	DATE OF INJECTION	WEIGHT	ARSENITE	MGMS. PER GRAM MOUSE	RESULT
			<i>grams</i>	<i>mgms</i>		
Feb. 14	Feb. 15	May 2	16	0.24	0.015	Died in 2 hours
Feb. 14	Feb. 15	May 2	15	0.225	0.015	Died in 6 hours
Feb. 14	Feb. 15	May 3	13	0.188	0.0145	Survived
Feb. 14	Feb. 15	May 3	10.5	0.152	0.0145	Died in 8 hours
Feb. 14	Feb. 15	May 9	14.5	0.21	0.0145	Died in 24 hours
May 16	May 17	May 31	20.5	0.3	0.015	Died in 24 hours

The first series of mice showed a clear cut difference of result after the administration of the same amount of arsenic and suggested that splenectomized mice were more resistant to arsenic poisoning than normal ones. The second series, however, made it plain that the difference, if any exists, must be within the limits of normal individual variation. The upper limit of endurance of the normal mice was above 0.014 mgm. of potassium arsenite per gram mouse and the lower limit of the lethal dose for splenectomized mice is below 0.0145 mgm. of potassium arsenite per gram mouse. Two guinea pigs, one of which had been splenectomized on the fourth of December received a



subcutaneous injection of potassium arsenite in a 1:100 dilution. Each received 0.0088 mgm. per gram of body weight. The normal animal weighed 532 grams and the splenectomized one weighed 599 grams. Both guinea pigs were very sick for 48 hours after which time the splenectomized one became better and by evening of the second day was entirely well; the normal one died about 54 hours after injection.

It may be concluded from these experiments that the body resistance to arsenic is not reduced by removing the spleen.

My thanks are due to Dr. B. Bernheim for assisting me with the surgical portion of the work.

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## THE CENTRAL ACTION OF CURARE.

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The peripheral action of curare on the motor nerve endings of striated muscle is so well known and the drug is so much used for this purpose in physiology and pharmacology that many are unaware of its central action. This central action has been studied on frogs especially by Wundt and Schelske (1), Magron and Buisson (2), Bezold (3), Vulpian (4) and by Tillie (5). They describe the action as similar to strychnine. In addition to these investigations on the general stimulating action, Sollmann and Pilcher (6) found that curare has a slight stimulating action on the vasomotor center. The present experiments were carried out mainly on dogs and show a definite stimulation of the central nervous system, but the type of stimulation differs markedly from the action of strychnine and can not correctly be referred to as a strychnine like action. The curare was injected directly into the cord or into the fourth ventricle with a hypodermic in the manner already described in the work with strychnine (7). The absorption is so slow from the cerebrospinal fluid the action centrally can be observed in detail before any general action due to the spread of the injected drug takes place. Incidentally we wish to state that this method may be used for the study of drugs on the vasomotor or respiratory systems and is the simplest method we know of and should give reliable results. This statement is based upon the fact that large doses of curare in the fourth ventricle never show the peripheral action of that drug, and strychnine and other drugs introduced in the same way show that absorption from the cerebrospinal fluid is slow.

In this work we used Merck's tested curare and the tested preparation of Dr. Theodor Schuhardt, Gorlitz. There was

no notable difference in the action of these preparations. The solution was prepared by dissolving the drug in water and filtering.

*Experiment I. June 26, 1916. Young dog, 7 kilos.*

- 9.30. Ether.
- 9.35. 1.5 cc. 0.25 per cent curare (Schuhardt) injected into the lumbar cord.
- 9.45. Animal recovered from the ether; no apparent action from the curare.
- 10.05. 0.5 cc. curare into the fourth ventricle; no anesthesia.
- 10.08. Emesis and defecation.
- 10.15. Spasm—somewhat resembling strychnine and hind legs more involved than the front. The legs do not always extend together as in strychnine poisoning and there is a rotation of the animal to the left around the long axis of the body. This seems to be a constant action of curare when administered in this region.
- 10.20. Strychnine like action in the hind legs; nothing in front.
- 10.30. Extension of the hind legs but no other action which resembles strychnine. The symptoms are more nearly those of camphor or picrotoxin. There is always a series of thrashing like movements, like those of a hooked fish, before the extensor spasm. There is no apparent action on the senses.
- 10.50. Respiration good, front legs paralysed, hind legs extended with more paralysis of the flexors than the extensors. Animal wags the tail when petted and can drink water. On way to recovery.
- 2.30. Animal in good shape. Some paralysis but recovered and was normal the following day.

*Experiment II. Dog. 5 kilos; a pup.*

- 10.15. Ether anesthesia and 2 cc. 0.25 per cent curare (Schuhardt) injected into the lumbar region.
- 10.20. Out of the ether, whines and bites at his hind legs. Apparently some sensory disturbance.
- 10.30. There is a decided action on the lower part of the body, none on the front part. Both motor and sensory parts effected. Dog bites viciously at the legs and lower part of the body. Reflexes are increased and the hind legs in a continuous jerky incoordinated movement.

- 10.40. The legs and tail jerk continuously, more behind than in front, some paralysis of the front legs; drug apparently spreading upward.
- 11.10. Animal dies from respiratory paralysis. This animal never showed the extensor action that those animals did in which the drug was injected into the 4th ventricle. There was simply a jerky incoördinated irritation like action with sensory disturbance and increased reflexes. The drug has some action on the lower cord but is more marked centrally.

*Experiment III. June 24.* The action of large doses of curare. Young dog, 6 kilos. No ether.

- 11.55. 2 cc. 0.5 per cent freshly prepared curare (Schuhardt) injected into the fourth ventricle. Immediately there is a spasm and a constant rotatory movement—wild, delirious and incoordinated. The eyes show a marked nystagmus and are widely dilated.
- 11.05. The spasm is changed into a running jerking movement, rabid, tries to run but is unable to do so. There is some asphyxia, perhaps. The spasm is as strong as some cases of strychnine but the legs fling and jerk separately like a camphor or a highly exaggerated physostigmine action. There is nothing to indicate a strychnine like action.
- 11.10. The animal is becoming fatigued. The twitching movements grow less and are much weaker; apparently some spread of the drug into the general system.
- 11.38. Animal dies; respiration stops before the heart.

*Experiment IV. June 28.* Dog, 6 kilos.

- 12.45. Injected 0.5 cc. of 0.5 per cent curare (Merck) into the region of the fourth ventricle. There was some blood drawn and apparently some of this solution went into the circulation directly.
- 2.20. There have been no symptoms up till this time and the animal seems normal.
- 2.20. 2 cc. clear cerebrospinal fluid withdrawn and 1 cc. 0.5 per cent of the same curare solution injected directly into the canal but in the direction of the tail. A clean cut operation.
- 2.30. Animal quiet; no apparent action. A little sleepy and heart somewhat irregular.
- 2.40. Reflexes of hind legs markedly increased. Tail in continuous fawning like involuntary movement becoming twitching or whip like.



- 2.45. A continuous tendency to move the hind legs, with constant tail movement.
- 2.50. Tail movement increases. Animal scratches head with hind feet. Some salivation and biting of the tail and feet.
- 2.55. Decidedly uncomfortable, irritable, reflexes very lively. The tail seems to be a source of annoyance to him. An apparent formication over the body.
- 3.00. Begins to snap the jaws and to lick the nose.
- 3.05. Miniature or petit spasm.
- 3.15. Spasm; petit.
- 3.20. Continuous indescribable movements seemingly due to general uneasiness with a spasm every few minutes. These spasms consist in a series of jerkings of the body backwards with the facial muscles twitching, ears back and to the center, tail in continuous whip like whirling motion so strong that it seems to wag the whole hind part of the animal. There is frothing at the mouth, snapping of the jaws, some respiratory difficulty and a throwing of the body backward. So far there has not been the strong spasm that caused the death in other animals. (See next experiment). During the intervals between these petit spasms the animal is in continuous movement with a superimposed physostigmine like tremor, especially of the hind legs.
- 4.20. The animal continues in the same state but apparently all effects of the drug are lessening in vigor. There does not appear to have been any disturbance of mentality at any time. Next day the animal is well.

*Experiment V. June 28, 1916. Healthy dog, 10 k'los.*

- 12.55. 1.5 cc. of 0.5 per cent curare (Merck) injected into the fourth ventricle after a like amount of clear fluid had been withdrawn.
- 1.00. Muscles around the eyes and ears twitching and some symptoms of fatigue.
- 1.05. Salivation, some scratching of the head with the hind legs.
- 1.09. Premonitory symptoms of spasms, twitching of the muscles of the head, frothing at the mouth and snapping of the jaws and turning the head strongly to the right.
- 1.10. Strong spasm, legs stiff but in running like separate movement hind legs drawn up to the body, some respiratory difficulties and continuous biting snapping movements, eyes dilated

and reflexes increased. The spasm lasts a minute and ceases apparently from fatigue.

- 1.15. Premonitory symptoms similar to those described.
- 1.16. Strong spasm with the symptoms the same as already described. These again last about a minute.
- 1.18. Premonitory symptoms.
- 1.20. Spasm in which the animal throws itself wildly about raising itself 60 or more centimeters from the floor, like a hooked fish; snaps and bites at itself and anything in the way; all of the muscles in strong separate jerky incoordinated rabid movements with an occasional twitch resembling strychnine, but typical only in that one is watching for a resemblance. The whole picture has no resemblance to strychnine action.
- 1.30. The spasm continues with intervals when it is not so pronounced. The head is in a decided oposthotonic condition, the tail curled strongly to the back and the hind legs drawn to the body, frothing at the mouth and snapping of the jaws. Eyes widely dilated. This condition quickly gives way to the running jerking throwing movements.
- 1.35. Animal chloroformed.

The action on frogs corresponds to the action recorded for dogs but the results are neither so definite nor so easily obtainable I laid bare the upper part of the spinal cord and medulla of 8 frogs and applied the curare solution by dropping it directly on the exposed part from a fine hypodermic needle. In some cases the action developed in a few minutes and more closely resembled the action of picrotoxin than strychnine although one can not describe it correctly as a picrotoxin action. The animals tend to jump and throw themselves as described in Experiments IV and V but after a few short spasms the paralytic action of the drug prevents further observations. Observation of the action on frogs in the main agrees with the action on mammals but is less definite.

I am aware that the composition of curare is indefinite and have no doubt that the observations of the other experimentators have in the main been correct. It is quite possible, however, that some samples of curare may contain a strychnine like acting drug. However, since the samples used by me have the

definite paralysing action of curare on the motor nerve endings and their central action is the same I attribute the statement of a strychnine like action more to the unfortunate choice of the experimental animal, than to differences in composition of the samples of curare.

#### RÉSUMÉ

Curare has a definite stimulating action when applied directly to the brain or cord. The time of the onset of the action varies with the dose. With a small dose as described in Experiment IV there may be no apparent action for twenty minutes or more. Then there is a noticeable increase in the reflexes and a tendency for the animal to be restless and to move about. There is apparently some action on the sensory nerves a feeling of formication, and as a consequence the animal licks and scratches the legs and body. Movement and restlessness increase and the licking and scratching give way gradually to biting and rabid actions. The development of the curare spasm is preceded by a twitching of the small muscles around the face and eyes, salivation and a sniffing or snorting movement, with some respiratory difficulty. The head is thrown back and the tail curled over the back. The animal turns the head strongly to one side (usually the right in my experiments though this may be due to the place of injection) and finally throws itself wildly in a rotatory extension spasm with the legs flinging wildly in all directions and purposeless. Some of the throws resemble the jumpings of a fish that has been caught on a line. As fatigue sets in the animal makes swimming like movements and there may be superimposed physostigmine like tremor. Death results from paralysis of the respiration. The effects cannot correctly be referred to as a strychnine like action.

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## THE SPONTANEOUS LIBERATION OF EPINEPHRIN FROM THE ADRENALS<sup>1</sup>

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It has been stated by various writers that epinephrin is liberated from the adrenals under experimental conditions in the absence of artificial stimulation of the splanchnics and that the liberation is dependent upon the integrity of these nerves. This liberation may be conveniently designated as spontaneous, without implying that it is necessarily a physiological process and not excited merely by the abnormal sensory stimulation, the anesthesia and other factors connected with the experiment.

Tscheboksaroff (1) injected blood collected from the adrenals of dogs after defibrination into small dogs and determined the effect on the blood pressure of blood obtained during stimulation of the major splanchnic with that of blood collected in the absence of stimulation and with the effect of blood obtained after section of both splanchnics. He concluded that section of the splanchnics causes distinct diminution of the adrenalin secretion.

O'Connor (2) using the Laewen perfusion preparation (frog's legs), states that citrate plasma collected from the adrenals in rabbits with the splanchnics intact has a decidedly greater constrictor effect than plasma collected after section of the splanchnics.

A] The observations of Trendelenburg (3) on the extreme rapidity with which a pressor action is developed in shed blood have shown how difficult it is to be certain that the vasoconstriction

<sup>1</sup> A preliminary note was published in the Proceedings of the Society for Experimental Biology and Medicine, May 24, 1916.

caused in the Laewen preparation by a given plasma is due to epinephrin. He finds that the pressor action is so quickly developed in blood on being drawn that citrate plasma cannot be used. For in the time required to centrifuge the blood considerable quantities of the vasoconstrictor substances are formed. Even entire citrate blood is already not fresh enough in a fraction of a minute after withdrawal.

In the present position of the question whether epinephrin is normally, or at least under experimental conditions, given off to the blood by the adrenals in the absence of artificial splanchnic stimulation, it seemed desirable to try methods less open to objection, especially so far as the determination of the amount of epinephrin liberated is concerned. As regards the further question whether after section of the splanchnics the discharge is completely abolished or only diminished, we do not see how it is possible to answer it by the aid of methods which permit the development of the pressor substances in the shed blood and depend upon vasoconstrictor reactions of the test objects.

We have endeavored to overcome this difficulty by using a method which does not require withdrawal of the blood to be tested, namely, collection of adrenal vein blood in a pocket of vena cava, which is then released. The presence of epinephrin in the blood is deduced from its action upon the denervated iris or nictitating membrane, and upon the blood pressure of the same animal. The identification of the change in the blood pressure curve produced by epinephrin is greatly assisted by simultaneous observation of the eye reactions. The amount of epinephrin liberated can be estimated by imitating the effect on the blood pressure curve by the injection of appropriate amounts of adrenalin in salt solution.

#### TECHNIQUE

Cats were employed in the great majority of the experiments. A few dogs were used for special points.

*The cava pocket.* Where the eye reactions are used alone the cava pocket need not be permanent. For certain purposes the temporary closing off of the pocket for a minute or two at a time is all that is

necessary, and in the interval the circulation proceeds practically in the normal way. A clamp is applied just above the iliac veins. The renal veins are then clamped and the segment of cava emptied of blood by gently stripping it upwards. Finally a clamp is put on the cava above the adrenal veins. Only a few seconds are occupied in the adjustment of these clamps. Small veins entering the cava segment have been previously tied. When the pocket is temporarily clamped off there follows a gradual dilatation of both pupils. On release of the pocket the epinephrin dilatation is easily seen in spite of the initial dilatation. But one advantage of the permanent pocket is that no change occurs in the pupil on clipping off the pocket.

When the blood pressure is studied as well as the eye reactions, the removal of the clamps from the temporary cava pocket generally produces too great a disturbance of the curve. A permanent pocket is therefore formed in the following way:

The coeliac and mesenteric arteries are first tied, then the abdominal aorta at the level of the kidneys. All small veins entering the cava segment are now ligated. Then the renal and lumbar veins are ligated. Meanwhile the blood has been draining out of the intestines and the hind legs. The legs are massaged so as to expel as much of the remaining blood as possible and a ligature is placed upon the inferior cava just above the junction of the iliac veins. The blood pressure is now high, and the animal usually lives a long time. It is kept very warm on a hot table, and the lower end is elevated so as to facilitate emptying of the cava pocket without manipulation. In some experiments the portal vein was tied, in addition, after the blood had well drained from the intestines. The only reason for tying the portal is to make sure that the necessary manipulation in adjusting the clamp on the upper end of the cava segment does not mechanically squeeze so much blood from the liver as to disturb the curve. In the great majority of experiments it was not found necessary or advantageous to tie the portal. The cava pocket thus formed represents only a blind pouch upon the circulation, the filling of which from the adrenal veins or the emptying of which after the removal of the upper clamp produces very little mechanical effect upon the blood pressure. In order to facilitate the application of the clamp a thick soft thread is tied in a loose loop around the cava above the level of the right adrenal. For certain purposes it is advantageous not to tie off the intestinal vessels, and by taking precautions against manipulation of the intestines during formation and release of the pocket, sufficiently



smooth blood pressure curves can be obtained without interrupting the circulation in the digestive tract. It is to be supposed that in investigations concerned with the study of the precursors of epinephrin or of the mutual influence of the adrenals and other abdominal organs, it would be advantageous to form the pocket in this manner. Where the eye reactions are alone being studied there is as already indicated no necessity for crippling any part of the splanchnic area. We have studied in a few animals the eye reactions only, in others only the blood pressure, but in the great majority both eye reactions and blood pressure.

*Method of measuring the rate of inflow of blood into the pocket.* While it is not necessary to know the quantity of blood entering the pocket in a given time to determine the amount of epinephrin given off in that time it is often desirable to estimate the concentration of the epinephrin in the blood. For this the rate of inflow must be obtained. This can be done, of course, by allowing blood to escape from the pocket as, for example, through a cannula in the left renal vein, and collecting the blood for a given time. Where only one or two observations of the rate of flow are required throughout the experiment this method suffices. To facilitate measuring the flow an indefinite number of times with approximate accuracy and without permitting the blood to escape or to come into contact with any foreign body, we have devised the following method: One of the iliac veins is tied near its distal end and the other near the cava. Both iliacs are then divided distal to the ligatures. By means of the ligature on the first iliac it is suspended vertically while the greater part of the cava segment lies undisturbed. The iliac vein thus serves as the neck of a measuring flask, so to say, the body of which is composed of the cava segment. It is not difficult to determine the moment when the blood entering the pocket practically without resistance, the walls of the vein being scarcely at all distended so long as the vertical portion of the pocket is empty, just reaches the proximal end of the iliac. The more rapid mounting of the blood in the relatively narrow iliac vein is easily seen. As the distal part of the cava segment is itself considerably narrower than the proximal, a fairly sharp reading can also be obtained by suspending the pocket without using the iliac vein. If undue exposure of the vein is prevented, a comparison of the flow from the adrenals in successive observations is made possible by comparing the intervals of time necessary for the pocket to fill up to this point. The quantity of blood required to fill the pocket can be determined once for all

in each animal. The vertical position of a portion of the pocket helps to empty it without manipulation when the clamp is removed.

TABLE 1

*Cat 61. Weight, 2.41 kg. Urethane. Cava pocket suspended to estimate time of filling*

NO. OF OBSERVATION	TIME	TIME OF FILLING OF POCKET IN SECONDS	BLOOD PRESSURE IN MM.
11-12	3.50	18	42
14		19	38
15		23	40
16		22	40
17		20	38
	4.30	Circulation was now much worse, the heart almost stopped.	
25		101	24
26		108	22
27		125	20

Now filled the pocket to the given level with Ringer's solution. In four successive observations the following quantities were required to fill the pocket; 0.4 cc., 0.35 cc., 0.4 cc., and 0.35 cc.

Left adrenal weighed 0.226 gram, and contained 0.20 mgm. epinephrin.

Right adrenal weighed 0.224 gram, and contained 0.19 mgm. epinephrin.

TABLE 2

*Cat 152. Weight, 1.85 kg. Urethane. Pocket suspended as described in technique. Cannula inserted in the left renal vein*

NO. OF OBSERVATION	TIME OF FILLING OF POCKET IN SECONDS	BLOOD PRESSURE IN MM.	RISE OF BLOOD PRESSURE
			mm.
4	45	70	8
5	48	66	7
6	45 with asphyxia	64	7-8
7	50 with asphyxia	56	
11	45	50	
12	47	50	
13	55	45	
14	58	45	
15	53	45	

Pocket was now allowed to fill to the given level, tied off and the blood determined by weighing. It amounted to 0.580 gram.

*Interpretation of the blood pressure tracings.* When eye reactions are available they are of great use in confirming the interpretation of a given rise in the blood pressure curve as an epinephrin rise. Of course where the blood pressure curve remains practically horizontal during the period of closure of the pocket a definite rise in the curve, commencing at a time interval after release of such a magnitude as is known to be associated with epinephrin reactions, can easily be identified as an epinephrin rise without simultaneous eye reactions. It is in the more irregular curves that the eye reactions are particularly valuable. As a rule, it is found that when the blood pressure is relatively low the closing off of the pocket produces little, if any, change in the height of the curve, and therefore the epinephrin rise subsequent to the opening of the pocket starts from a practically horizontal curve (see figs. 5, 6, 14). As already mentioned, even without eye reactions the blood pressure curve then gives perfectly definite proof of the liberation of epinephrin, although with low blood pressure the epinephrin rise is apt to be smaller for a given time of filling of the pocket than with a high blood pressure, since the quantity of blood collected is less. When the blood pressure is high the closing off of the pocket is usually accompanied by a more or less gradual drop of pressure, succeeded on opening the pocket by an immediate and abrupt rise. This initial rise is then followed at the interval appropriate to the epinephrin reaction by another rise (see fig. 10). The difference in the character of the curve associated purely with differences in the initial blood pressure at the time of closure of the pocket are well illustrated in figures 1 and 2. In observation 1, figure 1, with a blood pressure of 30 mm., the curve falls very little during period of closure of the pocket and remains horizontal after opening. In this animal spontaneous liberation of epinephrin was not taking place since one adrenal had been removed and the splanchnic supply of the other cut. In figure 2, observation 5, the initial blood pressure had been increased to 80 mm. by clipping off arteries. On closing the pocket, the curve falls distinctly. When it is opened there is an instantaneous abrupt rise, succeeded by a gradual small rise which brings the curve back to the initial level. No eye reactions whatever were elicited on opening the pocket, and the slight gradual rise does not present the characteristic features of an epinephrin rise.

In the absence of simultaneously elicited eye reactions the question might sometimes be puzzling to a novice in such observations, whether the epinephrin rise was not merely a continuation of the recovery of



the original pressure diminished by the closing off of the pocket. The epinephrin rise, however, by its character and time relations is, when at all considerable, practically always capable of being discriminated with certainty from other elevations which might be present on the curve even when the blood pressure curve shows a good deal of irregu-

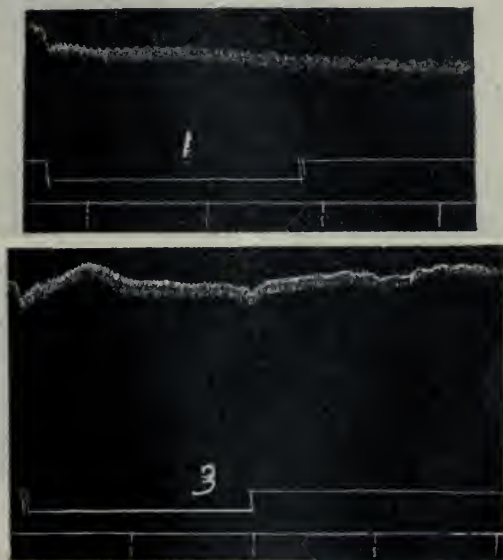


FIG. 1. CAT 81

1, Pocket, showing no liberation of epinephrin by the left adrenal whose splanchnic supply had been cut five weeks previously, and the right adrenal excised. 3, pocket experiment showing the same thing, but with a higher blood pressure, produced by clipping the abdominal aorta. In observation 1, as is generally seen with low blood pressure, the curve is less disturbed by closing and releasing the pocket than with a high blood pressure. In all the figures the time trace gives half minute intervals. The line of zero pressure is the upper signal line unless otherwise indicated.

larity, and in the absence of eye reactions. It is self evident that it is not possible to assay the amount of epinephrin liberated as accurately with an irregular pressure curve as with a regular one. And while extremely slight epinephrin rises can be surely distinguished when eye reactions are also available, a very small rise cannot in the absence of an eye reaction be taken as evidence of liberation of epinephrin,



unless as already stated the blood pressure curve remains practically horizontal during the period of closure of the pocket.

The epinephrin rise is very often preceded by a slight fall of pressure. When this is the case the pupil reaction commences synchronously

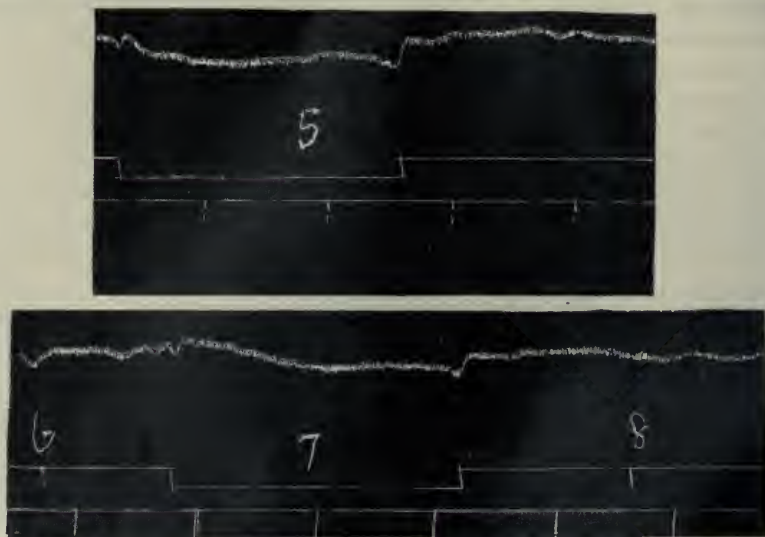


FIG. 2. CAT 81

Animal prepared by excision of right adrenal and section of nerves of left. The tracings illustrate the changes produced in the blood pressure curve by releasing the cava pocket when no epinephrin effect is present. Such changes can be easily discriminated from the effects produced by epinephrin. 5, pocket with left adrenal vein free. Blood pressure was higher than in observation 3, figure 1, and the effect of opening the pocket is correspondingly greater. 7, pocket with left adrenal vein clipped at 6. On releasing the pocket the usual immediate small rise in the blood pressure occurred. It is less pronounced than at 5, as the amount of blood in the pocket was less. Some blood found its way into the pocket in spite of the absence of the right adrenal, possibly through leakage past the lower cava clip. At 8, the clip was removed from the left adrenal and no epinephrin rise occurred. The line of zero pressure has been moved up 25 mm.

with the beginning of the fall (figs. 15 to 18). Elliott (5) states that the curve of blood pressure when the splanchnics are stimulated (the circulation in the splanchnic area not having been interfered with) shows a characteristic drop succeeding the immediate rise, "the cusp of the curve being always placed at the same time interval from the

beginning of the rise. The instant of the turn is that very moment when the nictitating membrane and the other structures of the denervated eye first move. The drop is paradoxically due to the liberation of adrenalin into the blood." In our observations with the cava pocket the same characteristic can often be noted and it undoubtedly affords a criterion of the beginning of the action of the spontaneously liberated epinephrin. When the blood pressure is low this preliminary drop is apt to be less marked (figs. 3, 4 and 14) than with a high blood pressure, or it may be absent (fig. 8). (Compare tables 11 and 12).

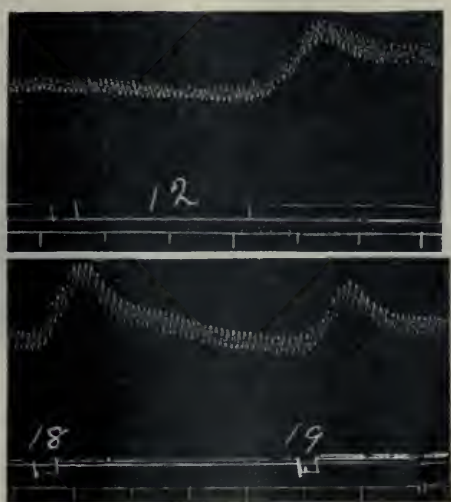


FIG. 3. CAT 116

12, Pocket experiment with stimulation of right splanchnic in abdomen after section of both splanchnics. Stimulation begun at the point indicated on signal line a short time after closure of the pocket. Good rise of pressure after opening the pocket due to epinephrin. 18 and 19, adrenalin injections to assay the amount of epinephrin liberated at 12. 18, 0.3 cc. of adrenalin (1:150,000). 19, 0.2 cc. of the same solution. Reduced to  $\frac{2}{3}$ .

The method, of course, permits the demonstration that epinephrin is liberated and the assay of its amount not only when the epinephrin is spontaneously discharged but also when it is set free in response to artificial stimulation of the splanchnics. In the latter case the quantity of epinephrin collected in the pocket being greater, the reaction obtained on release is also greater, so that a much more striking de-

monstration of the liberation of epinephrin by splanchnic stimulation is obtainable than in Asher's experiments (4) (see figs. 3 and 4). A smaller output of epinephrin can be detected and assayed in this way, than when the splanchnics are stimulated with the cava open. The experiment can also be made under more physiological conditions than with Asher's technique since, as already stated, it is not absolutely necessary for the pocket experiments to tie off the intestines; and the disturbance of blood pressure curve by the splanchnic stimulation is allowed to disappear before the pocket is opened. Certain other technical details may be mentioned here:

*Increasing the eye reaction by clamping alternative arterial paths.* The sensitiveness of the eye-reactions to injected adrenalin or to epinephrin discharged from the adrenals, for example, in response to stimu-

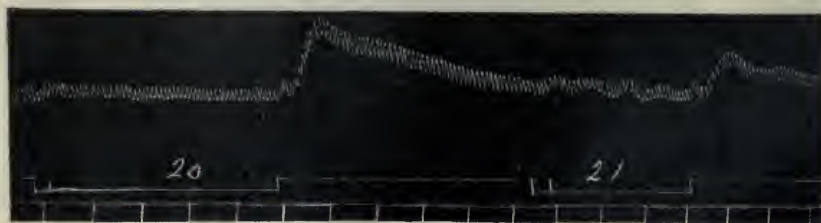


FIG. 4. CAT 116

20, pocket experiment with stimulation of right splanchnic in abdomen after section of both splanchnics 21, repetition of 20, but with a shorter time of stimulation. The epinephrin rise after 20 is considerably greater than after 21.  $\frac{2}{3}$  reduction.

lation of the splanchnics, can be increased notably by temporarily clamping off alternative arterial paths. This must be done at such an interval of time after the beginning of stimulation as is not more than sufficient to allow the epinephrin to reach the beginning of the aorta. A larger proportion of the blood containing the epinephrin is thus forced to take the path to the eye whose reactions are being studied. If, for instance, the left iris is the denervated one, clamping at the proper moment of the thoracic aorta and the innominate markedly increases the reaction. It can be further increased by tying off all accessible branches of the left carotid except those through which the eye must obtain its blood supply. The best demonstration of the increased effect on the eye of epinephrin discharged in response to splanchnic stimulation is obtained when the discharge is caused by a strong



stimulus lasting only a second or two. All this refers to the cat. In larger animals with a longer circulation time longer periods of stimulation could be employed. For, to intercept the first of the epinephrin, the arteries must be clipped in cats with a good circulation three or four seconds after the beginning of stimulation, and the clipping of the thoracic aorta, of course, interferes with the washing out of epinephrin already liberated into the adrenal capillaries.

It will be seen that except for the very small doses of adrenalin injected there is always a better reaction when alternative paths are clamped. Although there can be no doubt that the total quantity of adrenalin offered to the sensitive structures influences the amount of the reaction in an important degree, it may be assumed that the concentration is also important. Accordingly, below a certain concentration no increase might be expected in the reaction by increasing the amount of adrenalin-containing blood going through the eye.

The quantity of epinephrin which can reach the eye when a reaction is evoked by stimulating a splanchnic nerve, with all the vessels free, is so extraordinarily small that the suggestion is obvious that it might be possible to use the reaction as a test for epinephrin in extraneous liquids in which the concentration can only be very low. To do this it would be necessary to inject as near the eye as possible. A few experiments were made on this point.

Thus, in a dog weighing 13.8 kg., whose left superior cervical ganglion had been excised 24 days previously, injection of an adrenalin solution into the central end of the left superior thyroid artery caused retraction of the nictitating membrane in 5.6 seconds from beginning of injection. The same quantity of adrenalin solution injected into the central end of a femoral vein was followed by retraction of the nictitating in 12.4 seconds. In the cat, results on which are given in table 4, injection of 1 cc. of a 1:150,000 solution of adrenalin into a femoral vein caused moderate dilation of the pupil and retraction of the nictitating in 11.2 seconds. Injection of 0.5 cc. of the same solution by means of a syringe into the central end of the left carotid gave maximal dilatation of the pupil in 2.5 seconds. The heart stopped completely but was re-started by massage. The dilatation of the left pupil was very persistent. Subsequent injection of 1 cc. of 1:700,000 adrenalin into the carotid gave a maximal effect on the pupil in 1 second, while the same amount injected into the femoral vein caused movement of the nictitating in 16 seconds without pupil reaction, the pupil already being dilated to some extent. 0.5 cc. of the same solution



TABLE 3

*Condensed protocol of experiment on Cat 19. Weight 2.24 kg. Left superior cervical ganglion exercised 8 days before. Urethane. Adrenalin injected into femoral vein*

TIME		ARTERIES CLAMPED AFTER INJECTION	PUPIL DILATATION BEGINS IN
		<i>seconds</i>	<i>seconds</i>
11.48	0.3 cc. (1 : 430,000).....	.	8.4 moderate
11.49	0.3 cc. (1 : 430,000), aorta clamped.....	3	8 moderate +
	Repeated with similar result		
12.04	0.4 cc. (1 : 850,000).....		10.8 slight
12.05	0.4 cc. (1 : 850,000), aorta clamped.....	3	8.6 moderate
	Repeated with practically same result		
12.10	0.2 cc. (1 : 850,000).....		No reaction
12.11	0.2 cc. (1 : 850,000), aorta clamped.....	4	No reaction
12.23	Left splanchnic stimulated (9 cm. between coils), 4 seconds.....		9.2 moderate -
12.26	Left splanchnic stimulated (9 cm. between coils), 4 seconds, aorta clamped	4	8.2 good +
12.28	Left splanchnic stimulated (9.5 cm. between coils), 4 seconds.....		10 moderate -
12.30	Left splanchnic stimulated (9.5 cm. between coils), 4 seconds, aorta clamped...	4	9 good +
2.48	0.5 cc. (1 : 700,000).....		8 moderate
2.50	0.5 cc. (1 : 700,000) (right subclav., right carotid and aorta clamped).....	4	7.2 good +
2.51	0.3 cc. (1 : 700,000).....		10.2 slight
2.52	0.3 cc. (1 : 700,000) (vessels clamped as at 2.50).....	4	7.4 good +
2.53	0.1 cc. (1 : 700,000).....		No reaction
2.55	0.1 cc. (1 : 700,000) (arteries clamped as at 2.50).....	4	8.8 moderate +
3.15	0.1 cc. (1 : 1,400,000).....		No reaction
3.16	0.1 cc. (1 : 1,400,000) (arteries clamped as at 2.50).....	4	No reaction
3.25	0.5 cc. (1 : 2,900,000).....		No reaction
3.26	0.5 cc. (1 : 2,900,000) (arteries clamped as at 2.50).....	4	12.6 nictitating

*Circulation time femoral vein to carotid:*

2.0 cc. methylene blue injected, 3.0 seconds

0.5 cc. methylene blue injected, 3.2 seconds

0.5 cc. methylene blue injected, 3.4 seconds

Left adrenal weighed 0.211 gram and contained 0.38 mgm. epinephrin.

Right adrenal weighed 0.189 gram and contained 0.31 mgm. epinephrin.

TABLE 4

*Cat 20. Weight 3.2 kg. Left superior cervical ganglion excised 9 days before. Urethane. Adrenalin injected into femoral vein. Both splanchnics cut in thorax at beginning of experiment*

TIME		ARTERIES CLAMPED AFTER INJECTION	PUPIL DILATATION BEGINS IN
		<i>seconds</i>	<i>seconds</i>
1.34	1.0 cc. (1:1,400,000).....		No reaction
1.35	1.0 cc. (1:1,400,000), aorta clamped	3	No reaction
1.39	0.2 cc. (1:700,000).....		9 slight
1.40	0.2 cc. (1:700,000), aorta clamped.....	3	9.2 better
	Repeated last two observations, same result.		
3.00	0.4 cc. (1:300,000).....		10 moderate
3.01	0.4 cc. (1:300,000), aorta clamped .....	3	9 much better
3.40	0.3 cc. (1:300,000).....		No reaction
3.41	0.3 cc. (1:300,000), aorta clamped.....	3.5	9 fairly good
	Repeated above observations with same results.		
4.20	0.1 cc. (1:140,000).....		15 slight
4.22	0.1 cc. (1:140,000), right subclav., right carotid and aorta clamped.....	3	14.2 very good
4.25	Arteries clamped as at 4.22 but no injection.		No effect
	Repeated observations of 4.20 and 4.22, same results.		
4.35	Tied accessible branches of left carotid		
4.55	0.1 cc. (1:140,000).....		No reaction
4.56	0.1 cc. (1:140,000), all arteries clamped as at 4.22.....	3	12.2 good
4.58	0.05 cc. (1:140,000), arteries clamped as at 4.22.....	3.5	15.2 small reaction
5.00	0.05 cc. (1:140,000).....		No reaction
5.07	0.2 cc. (1:140,000).....		12 moderate
5.08	0.2 cc. (1:140,000), all arteries clamped	3.5	10.8 good
5.20	0.3 cc. (1:140,000).....		10.2 good
5.21	0.3 cc. (1:140,000), all arteries clamped	3.5	9.2 very good

Left adrenal weighed 0.169 gram and contained 0.22 mgm. epinephrin.

Right adrenal weighed 0.182 gram and contained 0.22 mgm. epinephrin.

gave good pupil and nictitating reactions in 3.2 seconds when injected into the carotid. 0.5 cc. of a 1:1,400,000 solution injected into the carotid, and even 0.25 cc. of the same solution, gave fair eye reactions.

The same was true when 0.3 cc. of a 1:3,000,000 solution of adrenalin was injected into the carotid. At this time 1 cc. of a 1:700,000 solution introduced into the femoral vein had no effect on the eye.

Of course, when injection is made into the carotid with a syringe the injection pressure is much more variable than when a burette, raised to a sufficient height, is employed. This accounts for the greater variability in the time intervals of the eye reaction in the experiment just quoted than in that given in table 5.

TABLE 5

*Injection of adrenalin into the carotid artery of a cat, by means of a burette*

	PUPIL DILATATION BEGINS IN
	<i>seconds</i>
0.4 cc. (1:700,000).....	4.8 good +, also nictitating.
0.2 cc. (1:700,000).....	3.2 good +, also nictitating.
0.3 cc. (1:700,000).....	4.8 good -, nictitating.
0.1 cc. (1:700,000).....	No reaction
0.5 cc. (1:700,000), into femoral vein....	8.8 moderate -

*Epinephrin assay.* For assaying the amount of epinephrin given off the blood pressure curve is, of course, better than the pupil dilatation. Still by determining the amount of adrenalin just needed to produce a given dilatation of the pupil, very fair results can be obtained with the pupil reaction also. To assay the epinephrin in the blood at a given period of an experiment it is necessary to make adrenalin injections while the conditions are still unchanged. The results of injections of adrenalin made early in an experiment cannot in general be used to estimate the epinephrin given off towards the end of the experiment, since, among other things, the blood pressure is usually higher in the earlier part of the experiment. It is scarcely necessary to add that we always assayed the adrenalin used. For this, and also for the assay of the adrenals, we employed the method of Folin, Cannon, and Dennis, (10) which we compared with the blood pressure method and found to correspond very closely.

The adrenalin solution was injected into the femoral vein when the cava pocket was only temporarily clamped off, into the external jugular when the cava pocket was permanent. It was determined by separate observations that the time interval after which the eye reactions appeared and their amount were sensibly the same whether a given quantity of adrenalin was injected through a cannula into the cen-

tral end of a femoral vein or through a catheter passed up through the femoral vein to the level of the adrenals. At least, this was found to be the case when the liquid ran in through the catheter at the same rate as through the femoral vein. When the orifice of the catheter was in such a position that the solution entered only slowly, the time interval of the eye reactions was, as might be expected, greater than with direct injection into the femoral vein. It was concluded that there was no advantage in point of accuracy in injecting by the catheter rather than into a vein. These observations are illustrated in table 6.

TABLE 6

*From an experiment on a 14 kg. dog, narcotized with urethane and ether*

	EYE REACTIONS
	<i>seconds</i>
2 cc. (1: 35,000), cannula.....	12.8
2 cc. (1: 35,000), catheter.....	14.0
2 cc. (1: 35,000), cannula.....	11.4
2 cc. (1: 35,000), cannula.....	14.2
3 cc. (1: 35,000), catheter.....	11.2
3 cc. (1: 35,000), cannula.....	11.0
4 cc. (1: 35,000), catheter (ran in slowly).....	15.0
4 cc. (1: 35,000), cannula.....	11.4
4 cc. (1: 35,000), catheter (ran in slowly).....	15.0
4 cc. (1: 35,000), cannula.....	13.0
4 cc. (1: 35,000), catheter (ran in freely as in vein injection).....	13.0

In one or two experiments we injected the adrenalin solution into the cava pocket through the left renal vein and then released the pocket (fig. 12).

#### THE SPONTANEOUS LIBERATION OF EPINEPHRIN

For convenience, as already stated, we speak of the epinephrin which is continuously given off under experimental conditions without artificial stimulation of the splanchnic nerves, as spontaneously liberated. In practically all the cats (nearly 40) used by us for these observations we obtained evidence of the presence of epinephrin in the cava pocket blood. We are not, however, able to decide definitely whether this liberation is a normal physiological process merely unveiled by the experiments or an abnormal process dependent on the necessary conditions



of the observations (anesthesia, unavoidable excitation of afferent nerves, etc. (2). The fact that the amount given off per unit of time in cats seems to vary within rather narrow limits even where the experimental conditions, particularly the kind and degree of anesthesia, vary considerably, might perhaps be interpreted as in favor of the first hypothesis. The increase

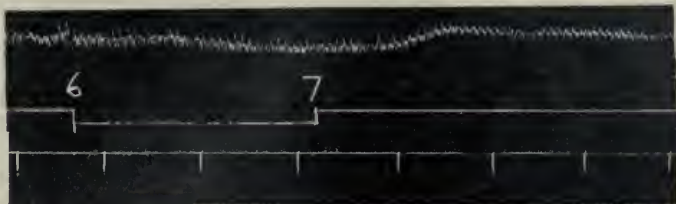


FIG. 5. CAT 57

6 to 7, Pocket experiment with epinephrin rise after release. Blood pressure is low, curve therefore smooth and even a small rise of pressure is capable of identification as an epinephrin rise. The preliminary drop in pressure is absent as is usually the case with low blood pressure.  $\frac{1}{4}$  reduction.

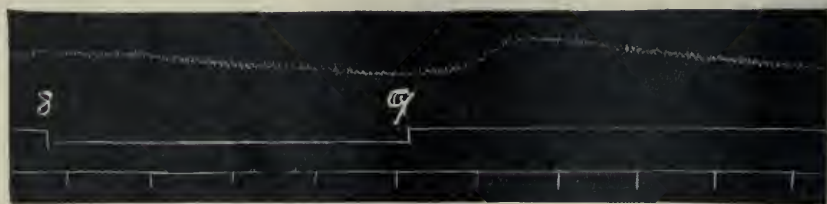


FIG. 6. CAT 57 .

8 to 9, Pocket. Epinephrin rise on release is greater than that in observations 6 to 7, fig. 5, as the duration of pocket 8 to 9 was greater, and therefore more spontaneously liberated epinephrin was collected.  $\frac{3}{10}$  reduction.

in the epinephrin effect upon the blood pressure on release of the pocket with increase in the time for which blood is collected in it is well shown in figures 5 and 6. The liberation is strictly associated with the integrity of the splanchnic nerve supply of the adrenals. At any rate, a discharge which has been perfectly capable of detection and even of assay with the nerves intact, falls at once below the threshold of detection by our methods as soon as the nerves have been divided. It is difficult

to believe that a process governed so definitely by a nervous mechanism has no physiological function. Whether some discharge still goes on after section of the splanchnic supply, could of course only be decided if more delicate methods were available. As already pointed out, positive conclusions based on the vasoconstrictor action of plasmas or blood perfused through frogs' legs cannot be allowed any weight where the quantity of epinephrin which can possibly be present is so small.

The results having been so consistent it is not necessary to multiply protocols. Figures 5 and 6 show that even where the rise of blood pressure is relatively small it may be perfectly definite. The tracings were taken in immediate succession from the same cat, but in figure 6 the period of closure of the pocket was approximately twice as long as in figure 5. It will be seen that the rise of pressure in figure 6 is also approximately twice as great. A condensed record of another experiment follows in table 7. Portions of the tracings of this experiment are given in figure 7.

The protocol of this experiment has been selected for reproduction because the animal had been so prepared that only one adrenal (the left) could liberate epinephrin, the splanchnic supply of the right having been previously cut. The perfectly clear demonstration of the liberation of epinephrin by the left adrenal when its vein was open to the pocket and the complete absence of the reactions when the left adrenal vein was clipped and only the right discharged into the pocket, are striking. As twice as much epinephrin would be given off by the two glands, it is clear that it cannot be a matter of difficulty when both are discharging, to demonstrate the epinephrin reactions. The experiment will be referred to in another connection in the discussion of the question whether after section of the nerve supply the adrenal ever regains the power to liberate epinephrin. Figures 8 and 14 further illustrate the fact that after section of the splanchnic supply of the adrenals the reactions due to the liberation of epinephrin, which were previously present, disappear entirely.

TABLE 7

*Condensed protocol of experiment on cat 137; weight 2.71 kg. Al nerve coming to right semilunar ganglion cut 11 days previously. Also the left superior cervical ganglion excised. Urethane, 4 grams by stomach tube. Permanent cava pocket with ligation of coeliac mesenteric and renal arteries and abdominal aorta. Both vagi cut. Cannula in carotid for blood pressure. Cannula in external jugular for adrenalin injection*

NO. OF OBSERVATION		DURATION OF CLOSURE OF POCKET IN SECONDS	PUPIL DILATATION IN SECONDS	RISE OF BLOOD PRESSURE	INITIAL BLOOD PRESSURE
				mm.	mm.
1	Pocket experiment.....	47	6.8	7	140
2	Clipped left adrenal vein.....				
3	Pocket experiment.....	45	No	No	
4	Adrenal vein released.....		Good 11.2	6	140
5	Pocket experiment.....	67	Good 6.2	9	145
6	Left adrenal vein clipped.....				
7	Pocket experiment.....	60	No	No	
8	Adrenal vein released.....		Good 9.8	7	
9	Pocket experiment.....	105	Very good 6.2	8	124
10	Ether given.....				
11	Pocket experiment during etherization.....	105	Fair 9	*	
12	Ether stopped.....				
13	Pocket experiment.....	105	Very good 9	13	80
15	Pocket experiment.....	105	Very good 7.2	10	96
18	0.3 cc. adrenalin (1:125,000) injected.....			18	88
19	0.3 cc. adrenalin (1:250,000) injected.....			12	80
20	0.2 cc. adrenalin (1:250,000) injected.....			8	82
21	Pocket experiment.....	105	Good 10	8†	
22	Clipped left adrenal vein.....				
23	Pocket experiment.....	130	No	No	
24	Adrenal vein released.....		Small reaction	7	
			20		

Left adrenal weighed 0.260 gram and contained 0.18 mgm. epinephrin.

Right adrenal weighed 0.286 gram and contained 0.25 mgm. epinephrin.

\* The blood pressure curve was spontaneously rising after the drop of pressure due to the ether so that although an epinephrin rise was indicated upon the curve its amount could not be measured.

† The assay of the epinephrin liberated in observation 21 works out at 0.00015 mgm. per minute per kg. of animal for one adrenal. Earlier in the experiment as large a rise of pressure was obtained with a considerably shorter duration of the pocket so that it is probable that when the circulation in the animal was better the liberation of epinephrin per minute was greater than this.



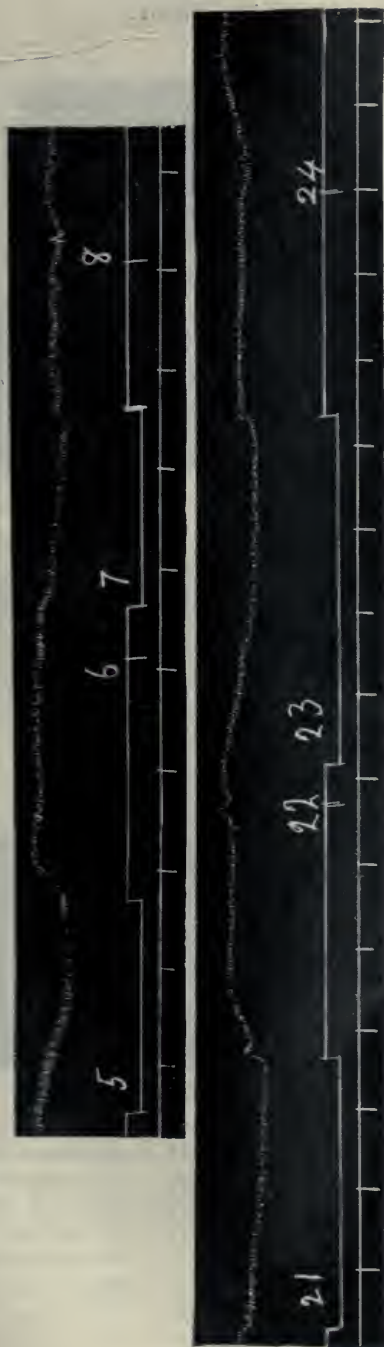


FIG. 7. CAT 137

The tracing illustrates the fact that no spontaneous liberation of epinephrin by the right adrenal after section of its nerves 11 days before could be demonstrated. 5, pocket experiment with both adrenal veins free, definite epinephrin effect with characteristic preliminary small drop of pressure. 7, pocket experiment with left adrenal vein clipped. The clip was put on at 6. No epinephrin effect on release of the pocket. 8, removal of the clip from the left adrenal vein followed by small epinephrin effect. 21, pocket experiment showing liberation (spontaneous) by the left adrenal with a lower blood pressure than at 5. 23, pocket with left adrenal vein clipped at 22. No epinephrin effect on release of the pocket, the pressure merely rising instantaneously to its permanent level and the curve then remaining horizontal till the clip was removed at 24 from the left adrenal vein, when a moderate epinephrin effect occurred. Line of zero pressure has been moved up 55 mm. for the portion of the tracing from 5 to 8 and 25 mm. for the portion from 21 to 24. To get blood pressure add 110 mm. and 50 mm. respectively.  $\frac{1}{3}$  reduction.





FIG. 8. CAT 117

4 to 6, pocket experiment with stimulation of both sympathetics in the thorax above the diaphragm. Stimulation begun at 5; stopped at 6. Pocket opened at 6. 13, pocket experiment after division of major splanchnics in abdomen and sympathetics in thorax. No epinephrin effect now seen on the blood pressure curve after opening the pocket. 14 to 17, pocket experiment with stimulation of sympathetics in thorax after division of major splanchnics in abdomen. Stimulation began at 15, stopped at 16. No evidence of liberation of epinephrin.  $\frac{1}{4}$  reduction.

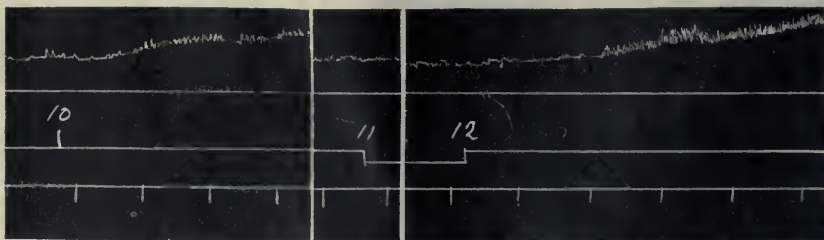


FIG. 9. CAT 57

10, 0.25 cc. adrenalin (1:80,000) injected. The heart had almost stopped but began to beat well again after the adrenalin injection. At 11 to 12, when the heart had again almost stopped and the blood pressure had fallen, a pocket experiment was made.  $2\frac{1}{2}$  minutes of the pocket period between 11 and 12 not reproduced to save space. After release of the pocket at 12, the heart was stimulated precisely as by the adrenalin and the blood pressure rose. Line of zero pressure is horizontal line nearest tracing.  $\frac{2}{3}$  reduction.

Figure 9 is reproduced because it shows in an interesting way the absolute similarity of the action of injected adrenalin and spontaneously liberated epinephrin. At this stage in the experiment (cat 57) the blood pressure had fallen to an extremely low point and the heart was almost stopped. The injection of 0.003 mgm. of adrenalin at observation 10 caused a marked recovery of the heart and a rise of blood pressure. In the next observation the pocket was closed for  $2\frac{1}{2}$  minutes between 11 and 12. On release of the pocket the heart, which in the interval between 10 and 11 had again almost stopped, began to beat well, and the blood pressure rose just as after the adrenalin injection.

*Quantitative results.* The quantities of epinephrin spontaneously liberated, as estimated by the injection of adrenalin in a number of experiments, are given in table 8. In different experiments the amount per minute per kilo of animal, varied from 0.0003 to 0.001 mgm. with both adrenals discharging. As it is not certain that the rate of discharge has any simple relation to the body weight, the total amount liberated per minute is also given. It varied from 0.0008 to 0.0028 mgm. In one experiment the discharge from a single adrenal was 0.0005 mgm. per minute or 0.00015 mgm. per kilo of body weight per minute. O'Connor by the frog perfusion method obtained in rabbits results ranging from 0.00014 to 0.0007 mgm. per minute per animal.

*The effect of section of the splanchnics is not due to the fall of blood pressure.* As in animals in which the circulation through the intestines has not been interrupted section of the splanchnic causes a decided drop in blood pressure, it might be asked whether it is not the interference with the blood flow through the adrenals consequent on this fall of pressure which is responsible for the inability to liberate epinephrin. Although in experiments in which the abdominal aorta, the mesenteric and the coeliac arteries have been tied, section of the splanchnics does not cause any considerable fall of pressure, while still abolishing the liberation of epinephrin, the objection was fully taken account of in a number of experiments in which only the right sympathetic

TABLE 8

NUMBER OF ANI-MAL	NUMBER OF OB-SERVATION	BODY WEIGHT KG.	DURATION OF POCKET IN SE-CONDS	INITIAL BLOOD PRESSURE IN MM.	TOTAL EPINEPHRIN IN MG.	TOTAL PER MINUTE	EPINEPHRIN PER KG. PER MINUTE	BLOOD IN POCKET IN GRAMS	ASSAY BY ADRE-NALIN	CONCENTRATION OF EPINEPHRIN
57	11-12	1.83	180	12	0.003	0.001	0.0006	0.308	0.25 cc. 1: 75,000	1: 1,000,000
59	9	2.36	90	90	0.0017	0.0011	0.0003	0.306	more than 0.25 cc. 1: 150,000	1: 1,800,000
81	27	2.65	Massage 120	52	0.0016	0.0008	0.0003*		0.2 cc. 1: 125,000	
95	22-25	3.275	105 with asphy- xia	140	0.0048	0.0028	0.0008	1.32	0.6 cc. 1: 125,000	1: 3,000,000
	26		105	130	0.0048	0.0028	0.0008		0.6 cc. 1: 125,000	1: 3,000,000
114	17	2.435	120	120	0.004	0.002	0.0008	1.255	0.5 cc. 1: 125,000	1: 3,100,000
	18-21		120 with brachia stim.	120	0.004+	0.002+	0.00085		More than 0.5 cc. 1: 125,000	
	22		120	120	0.004	0.002	0.0008		0.5 cc. 1: 125,000	
	23-26		120 with asphy- xia	118	0.004+	0.002+	0.00085		More than 0.5 cc. 1: 125,000	
	29-32		120 with asphy- xia	120	0.0045	0.0022	0.0009		More than 0.5 cc. 1: 125,000	1: 2,700,000
	34		120	116	0.005	0.0025	0.001		Less than 0.65 cc. 1: 125,000	
	35-38		120 with asphy- xia	114	0.005	0.0025	0.001		Less than 0.65 cc. 1: 125,000	

\* One adrenal only.

TABLE 8—Continued

NUMBER OF ANI-MAL	NUMBER OF OB-SERVATION	BODY WEIGHT KG.	DURATION OF POCKET IN SE-CONDS	INITIAL BLOOD PRESSURE IN MM.	TOTAL EPINEPHRIN IN MG.	TOTAL PER MINUTE	EPINEPHRIN PER KG. PER MINUTE	BLOOD IN POCKET IN GRAMS	ASSAY BY ADRE-NALIN	CONCENTRATION OF EPINEPHRIN
116	2	2.76	90	52	0.0033	0.0022	0.0008	1.01	0.5 cc. 1: 125,000	1: 3,000,000
	12		90 with stim. of rt. splanchnic	50	0.0021	0.0014	0.0005	†	0.3 cc. 1: 125,000	1: 5,000,000
	13		90 with stim. of rt. splanchnic	50	0.0021	0.0014	0.0005	†	0.3 cc. 1: 125,000	1: 5,000,000
137	21	2.71	105	140	0.0008	0.0005—	0.00015*		0.2 cc. 1: 250,000	

\* † This represents secretion from the right adrenal alone during stimulation of right splanchnic. Before observations 12 and 13 both major splanchnics were divided.

\*One adrenal only.

including the major splanchnic was divided in thorax. In some of these experiments section of the right splanchnic was made at the time when the pocket observations were being carried out. In others the connections of the right semilunar ganglion including the major splanchnics were severed beforehand and the animal allowed to recover. When the left adrenal vein was clipped the blood collected in the cava pocket gave no evidence of epinephrin. Removal of the clip after the opening of the pocket is usually followed by epinephrin reactions. The rise of blood pressure following removal of the clip from the left adrenal vein, although it may sometimes be as great as with an ordinary pocket experiment made only a little time before or after, is more gradual and the pupil reaction comes after a somewhat longer time interval. (See for example table 7, observation 21, as compared with observations 22 to 24, and figure



7.) The simplest interpretation of this is that the epinephrin accumulated during occlusion of the adrenal vein, instead of passing at once along the cava as the epinephrin-containing blood collected in the pocket does, must be more gradually washed out of the adrenal vessels. This interpretation is corroborated by the observation that when the adrenal vein is clipped and a cava pocket formed before the clip is removed, so that the accumulated epinephrin-containing blood in the adrenal now escapes into the pocket, the rise of blood pressure and eye reactions elicited on releasing the pocket occur at the same time interval, and have the same character as when the blood is collected in the pocket with the adrenal vein free.

The magnitude of the epinephrin effects obtained by releasing the adrenal vein after a period of occlusion is usually less than when the adrenal vein blood is collected in the cava pocket for the same length of time. This is to be expected. It is, indeed, surprising that the relatively small amount of blood which can be pent up in the adrenal should sometimes contain as much or nearly as much epinephrin as the much larger quantity of adrenal blood which is collected in the same time when the vein is discharging freely into the pocket. An average cava pocket in a cat will contain about 1 gram of blood, or more than double the combined weight of the two adrenals, so that the amount of blood in an adrenal, even when passively congested by clipping its vein, can only be a small fraction of the amount which it discharges into the pocket with the vein free. The concentration of epinephrin must be much greater in the blood behind the adrenal vein clip than in the blood when collected in the pocket. In other words, the amount of epinephrin liberated is not proportional to the quantity of blood flowing through the gland but depends also on the time. If epinephrin is liberated steadily at a fairly constant rate the concentration in the adrenal vein blood must vary inversely with the rate of flow.

Table 9 illustrates the results obtained in one of the acute experiments in which the right sympathetic was divided in the thorax a little above the diaphragm. Portions of the trac-

TABLE 9

*Condensed protocol of experiment on cat 37. Weight, 2.45 kg. Left superior cervical ganglion excised 10 days before. Temporary cava pocket method used as described under technique.*

NO. OF OBSER- VATION	TIME		DURATION OF POCKET IN SECONDS	PUPIL DILATATION IN SECONDS	BLOOD PRES- SURE RISE		INITIAL BLOOD PRESSURE
					Milli- meters	Begins in seconds	
	9.15	Urethane 4 grams by stomach tube.....					
	10.00	A little ether. Cava pocket prepared. Cannula in carotid and trachea.....					
3	11.35	Pocket experiment....	30	Very good 9.2	22	8	110
4	11.40	Pocket experiment with left adrenal clipped.....	90	Very good 12.8	13	9	100
6	11.43	Pocket with left adre- nal vein clipped.....	60	15	5	10	80
7		Removal of adrenal vein clip.....			6		64
	11.50	Thorax opened. Cut right sympathetic above diaphragm....					
	11.55	Clamped abdominal aorta.....					
11	12.00	Pocket experiment with left adrenal vein clipped.....	90	No	No		48
12		Released adrenal vein..		Slight	Slight		44
14	12.05	Pocket experiment....	75	Very good 22.6	14	20	40
15	12.30	Pocket experiment with left adrenal vein clipped and stimulation of right splanchnic for 2 min- utes (5 secs. on and 5 secs. off).....	150	Very good 32	18	20	40
17	12.40	Pocket experiment with left adrenal vein clipped.....	90	No	No		
18		Removed adrenal clip..		Slight	10	12	54

TABLE 9—Continued

NO. OF OBSER- VATION	TIME		DURATION OF POCKET IN SECONDS	PUPIL DILATATION IN SECONDS	BLOOD PRES- SURE RISE		INITIAL BLOOD PRESSURE
					Milli- meters	Begins in seconds	
19	12.45	Pocket experiment. ....	90	Good 20.2	13	15	58
	2.15	Left sympathetic di- vided in thorax above diaphragm. ....					
35	2.20	Pocket experiment with stimulation of right and, occasion- ally left sympathet- ics for 3 min., (5 secs. on, 5 secs. off).....	180		14	26	34
38	2.40	Pocket experiment with massage of both adrenals. ....	120		18	45	29

Left adrenal weighed 0.197 gram and contained 0.14 mg. epinephrin.

Right adrenal weighed 0.201 gm. and contained 0.16 mg. epinephrin.

ings from this experiment are reproduced in figures 10, 11 and 12. Results in animals in which the splanchnic supply of the right adrenal was divided in advance of the acute experiment have already been quoted in table 7 (fig. 7). They show clearly that the right adrenal no longer discharges a detectable amount of epinephrin. For when the left adrenal vein is clipped, blood collected in the cava pocket produces no reaction (fig. 10), whereas with the left adrenal vein free a good reaction is obtained. When the nerve supply of both adrenals has been cut, pocket experiments are negative, although epinephrin in good amount is liberated by stimulation of the splanchnics (fig. 11) and by massage of the glands (fig. 12)

*Does the denervated adrenal eventually regain the power of liberating epinephrin?* It is known that cats survive indefinitely when one adrenal is removed and the splanchnic supply of the other cut. If epinephrin has a physiological function, or at any rate an indispensable one, it must be supposed that eventually, even in the absence of innervation, it will be given off from the denervated glands. Elliott (5) speaks of this as something self-



FIG. 10. CAT 37.

14, Pocket experiment showing spontaneous liberation of epinephrin by the left adrenal, the right being eliminated by previous section of the right splanchnic in the thorax. 17, pocket experiment with left adrenal vein clipped. No epinephrin effect on release, till the clip was removed at 18 from the left adrenal vein, when after the usual interval a distinct epinephrin rise occurred due to the epinephrin pent up in the adrenal by the clip. 19, pocket experiment, with left adrenal vein open. Good epinephrin rise.  $\frac{1}{10}$  reduction.



evident. "Ultimately," he says, "the glands must be capable of automatic excretion, for the decentralized gland suffices to keep the animal alive." On testing the question, however, we find no evidence that epinephrin in detectable amount is liberated from the adrenals of cats even a considerable time after the innervation has been destroyed.

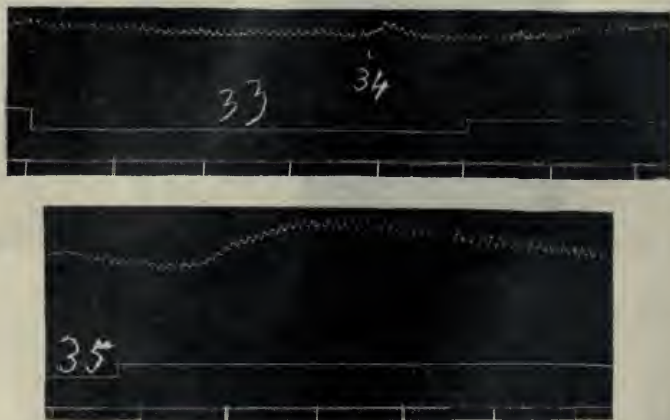


FIG. 11. CAT 37

33, Pocket experiment, after section of both sympathetics in the thorax above the diaphragm. No epinephrin effect on release, the curve rising gradually to the original level and then becoming horizontal just after the end of the portion of the curve we reproduce. 34, elevation of blood pressure curve due to spontaneous movement of the animal. 35, pocket experiment, with stimulation of right sympathetic trunk in thorax. The pocket was closed for three minutes, only the last twenty seconds of which are shown on the tracing. The tracing shows that the right adrenal although liberating no detectable epinephrin spontaneously after section of its nerves is capable of liberating a considerable amount when these nerves are stimulated. Reduced to  $\frac{1}{4}$ .

The right adrenal was removed from a cat (no. 81) and the fibers coming to the left semilunar ganglion divided. The left superior cervical ganglion had been excised ten days previously. Five weeks after removal of the adrenal, pocket experiments were made, with an absolutely negative result as regards epinephrin reactions on the blood pressure or the eye (fig. 1). Stimulation in the course of the major splanchnic in the abdomen on the left side was also negative. The gland,

however, contained plenty of epinephrin capable of being discharged into its blood vessels, as was shown by massage observations (fig. 13). For example, in observation 27, massage was practiced for two minutes with the cava pocket closed. The rise of blood pressure, accompanied by very good eye reaction 8 seconds after release of the pocket, corresponded to a liberation of 0.0016 mgm. of epinephrin (see table 8).

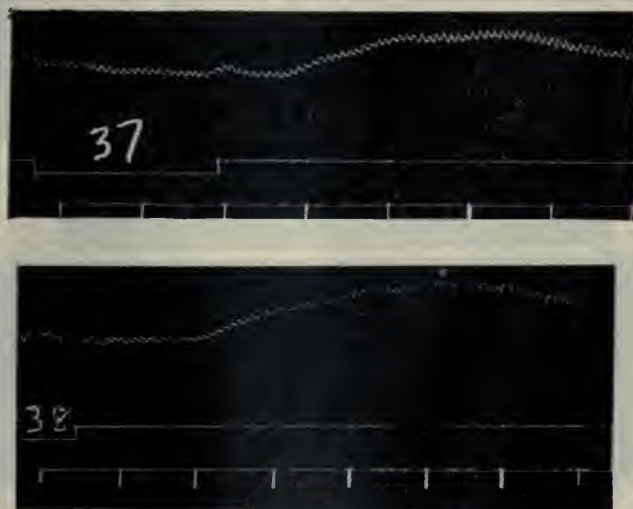


FIG. 12. CAT 37

37, injection into cava pocket of 0.5 cc. of 1:40,000 adrenalin. Pocket released in the usual way in order to compare the effect of the adrenalin with the effect of the epinephrin liberated in pocket experiment 38 by massage of the adrenal. The pocket was closed and massage kept up for two minutes, only the last twenty seconds of which are shown on the tracing. The splanchnic supply of both adrenals had previously been cut. Reduced to  $\frac{2}{3}$ .

We have shown the same thing in a different way by dividing the nerve supply of the right adrenal, and then, after the animal has recovered, making pocket experiments with the left adrenal vein alternately free and clipped. When the left vein is allowed to discharge into the pocket distinct evidence is obtained of the presence of epinephrin in the blood released, after the clamp is removed from the pocket. But when the left

adrenal vein has been previously clipped, the blood collected in the pocket from the right adrenal produces not the slightest epinephrin effect either on the blood pressure or on the eye. This is not due to the smaller amount of blood collected in a given time. For the negative result is in no wise altered if the period of collection is lengthened. Also, on now sectioning the splanchnic supply of the left adrenal, although the pocket fills as rapidly as before with both adrenal veins free, there are no epinephrin reactions (see tab e 7, fig.7 (cat 137, observations

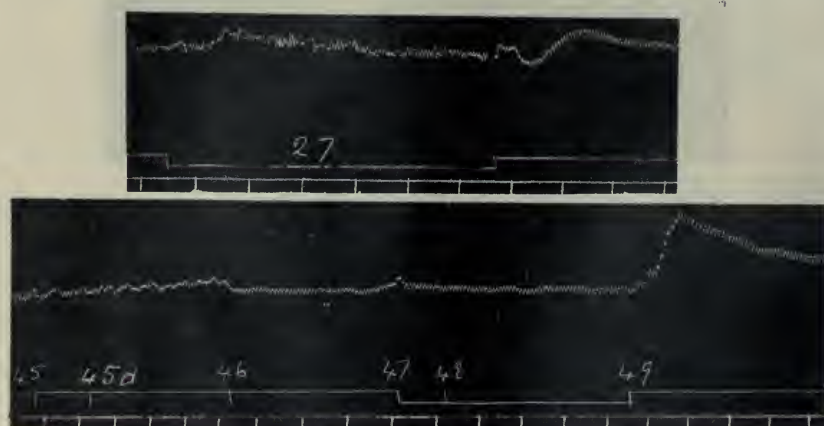


FIG. 13. CAT 81

Showing the effect of massage in liberating epinephrin from the left adrenal whose nerves had been cut 5 weeks before. 27, cava pocket with massage, the left adrenal vein being open to the pocket. 47 to 49, pocket with massage, the left adrenal vein having been closed at 45 and opened at 48, after closure of the pocket. Massage begun at 45-A, stopped at 46. Reduced to  $\frac{2}{3}$ .

5 to 8 and 21 to 24) ). Of course, all that can be deduced from these negative results is that if any epinephrin is spontaneously liberated from the denervated glands, its amount is too small for detection. It was shown that epinephrin could be discharged from the glands under massage in pocket experiments.

Evidence has already been brought forward that considerable amounts of epinephrin can be liberated into the adrenal vessels when the outflow of blood from the gland is prevented. This is the case both for the spontaneous liberation of epinephrin



and for its liberation by splanchnic stimulation. In some of the massage experiments an attempt was made to test the further question whether the epinephrin once liberated into the adrenal vessels can lie there for some time without losing its effect. Massage was practiced in observations on cat 81 with the left adrenal vein clipped. An interval was then allowed to elapse after closure of the cava pocket before the adrenal vein was freed. On opening the pocket good epinephrin reactions were obtained (see fig. 13 (cat 81, observations 45 to 49) ) associated with a very good pupil reaction in 9.8 seconds. In another experiment on the same animal the left adrenal vein was clipped, the cava pocket then closed and massage of the left adrenal practiced for two and one-quarter minutes. The pocket was now released the adrenal vein being still clipped. No epinephrin reactions either on blood pressure or eye were obtained. After an interval of  $1\frac{1}{2}$  minutes the cava pocket was again closed, the adrenal vein then released and the cava pocket allowed to fill for  $2\frac{1}{4}$  minutes without further massage. On release of the pocket excellent eye reactions (pupil and nictitating) and a good rise of blood pressure were obtained.

Either then the epinephrin liberation is not indispensable, or the necessary physiological supply of epinephrin is too small to be detected by methods which clearly detect the epinephrin liberated through the splanchnic nerves under experimental conditions, and also the epinephrin liberated by massage of adrenal glands long deprived of their innervation.

*Nature of the spontaneous epinephrin liberation.* If good evidence could be adduced that epinephrin is necessary for survival and for health, the question raised on a previous page as to the nature of the epinephrin liberation seen under experimental conditions would seem to be answered by the observations just described—and in this sense, that it is not a discharge of the same order of magnitude as the physiological liberation but a greatly accelerated discharge connected with stimulation of the nervous mechanism controlling the secretion by the abnormal irritation of sensory nerves, or by some action of the anesthetic. If, however, the discharge of epinephrin is essentially



an "emergency function," as suggested by Cannon, it may be that health can be maintained without liberation of epinephrin or with too small a liberation to be detected, although the animal may be handicapped in circumstances which normally evoke the emergency secretion. The increased discharge under experimental conditions might itself be considered an emergency secretion. However this may be, it was seen that cats, with one adrenal removed and the nerves of the other cut, behaved in the same way as cats with intact adrenals, in regard to certain signs of emotional disturbance supposed by some writers to be associated with epinephrin liberation. When the animal was frightened or rendered angry the pupil of the denervated eye dilated well, and dilatation began practically at once, that is to say, much sooner than the same reaction when it is known to be elicited by epinephrin. The dilatation was sometimes as great as, or even greater than the normal eye. In one cat it was constantly greater. The relative amount of dilatation of the pupil in the denervated eye as compared with its fellow was at least as great in all the animals tested as in cats with intact adrenals. The pilomotor effects were not less than in normal cats. In asphyxia and ether anesthesia the pupil of the denervated eye in the cats without adrenal innervation became wider than the pupil of the other eye.

Another point which has some bearing on the question of the nature of the spontaneous liberation of epinephrin seen under experimental conditions, may be mentioned. We have produced evidence in a previous paper (6) that a portion, and usually a very considerable portion, of the epinephrin liberated by electrical stimulation of the splanchnics must be newly formed epinephrin, and cannot have come from the stock in the glands at the beginning of the experiment. The same thing seems to be true of the spontaneous liberation, although, with the nerves intact, there is always a much more definite "spontaneous" loss of epinephrin from the store under experimental conditions than when the cut nerves are stimulated electrically, despite the fact that more epinephrin passes into the blood in a given time with electrical stimulation of the nerves.

Thus, if in cat 37 (protocol, table 9) the discharge from the left adrenal between 11.50 a.m. and 2.20 p.m. was at the smallest rate found in our experiments for one adrenal, namely 0.0005 mgm. per minute, this would amount to 0.075 mgm. for the two and a half hours. The nerves of the right adrenal were cut at 11.50 and those of the left at 2.20. It has been shown by Elliott that section of the nerves protects the store of epinephrin in a gland from discharge. At the end of the experiment the left adrenal contained only 0.02 mgm. of epinephrin less than the right.

In cat 137 the left adrenal lost from its store 0.07 mgm. in 5 hours or over 0.0002 mgm. per minute. The gland discharged spontaneously (see table 8), 0.0005 mgm. per minute.

In cat 116 the major splanchnics were divided after the experiment had proceeded for four and one half hours. The epinephrin liberated before division was estimated at 0.002 mgm. per minute (table 8). If the liberation was at the same rate, say for 4 hours, the amount discharged by the two adrenals would be 0.48 mgm. At the end of the experiment the left adrenal contained 0.11 mgm. and the right 0.09 mgm. The highest content of an adrenal in cats suddenly killed does not exceed 0.35 to 0.38 mgm. Even if the adrenals in this animal at the beginning of the experiment had a maximal load, not less than half the amount discharged must have been new formed.

If the spontaneous discharge is associated with new formation of epinephrin it ranges itself, so far as this fact goes, with the genuine secretions.

*Do the major splanchnics carry all the fibers concerned in the liberation of epinephrin?* It has been shown by Elliott (5) that section of the major splanchnics alone does not suffice to protect the adrenal (in the cat) from discharge of its epinephrin store. We have found that this is true also for the dog. The question arises whether the major splanchnic carries all the fibers which control the spontaneous liberation of epinephrin, and also, what is probably the same thing, whether it carries all the fibers, artificial stimulation of which causes liberation of epinephrin. We have tried to test this in a number of experiments one of which is illustrated in table 10. Cava pocket observations were made with stimulation of one or both sympathetics in the thorax just above the diaphragm.

The epinephrin reactions obtained on release of the pocket were noted. Then the major splanchnic was divided in the abdomen and stimulation of the sympathetics repeated, with the cava pocket closed for the same or for a longer period. Positive epinephrin reactions on release of the pocket would now, of course, indicate that a portion of the efferent nervous path concerned in the liberation had not been divided. In other observations the spontaneous liberation of epinephrin was first verified by pocket experiments before division of any nerves. The major splanchnics in the abdomen were then cut, and it was noted whether blood collected in the cava pocket still caused any sensible epinephrin reaction. In one experiment (cat 95, see protocol, table 12) we obtained a definite, though small, rise of blood pressure after release of the pocket when the major splanchnics had been previously cut. The rise was associated with a slight pupil reaction. On now dividing the fibers coming to the semilunar ganglion on both sides and repeating the experiment, the result was negative. Before division of the major splanchnics good epinephrin reactions had been obtained. It need not be assumed that after division of such a very important fraction of the total innervation as that carried in the major splanchnic, all cats will show a definite spontaneous liberation of epinephrin. As a matter of fact, we have also seen the opposite result. For instance, in cat 116 (fig. 14, observations 2 to 9) no detectable epinephrin was given off after division of both major splanchnics in the abdomen. Before division of the major splanchnics the amount of epinephrin spontaneously liberated in this animal (at observation 2) was assayed (by means of adrenalin injections such as those shown in the figure in observations 5 and 6) at 0.0008 mgm. per minute per kilo of animal, an amount of the usual magnitude at least. The major splanchnics were then cut, and observation 9 shows no trace of liberated epinephrin. The glands were still at this time perfectly capable of excreting epinephrin. For stimulation of the right splanchnic (fig. 3, observation 12) while the pocket was closed, caused considerable liberation, as shown by the marked rise in blood pressure and eye reactions (pupil, nictitating and



TABLE 10

*Condensed protocol of experiment on cat 117. Weight, 1.955 kg. Left superior cervical ganglion excised 26 days previously. Urethane, 4 grams Prepared permanent cava pocket with ligations of arteries. Isolated both sympathetic trunks in thorax above diaphragm without ligating them*

NO. OF OBSERVATION		DURATION OF POCKET IN SECONDS	PUPIL DILATA- TION IN SECONDS	BLOOD PRES- SURE RISE		INITIAL BLOOD PRESSURE
				Milli- meters	Begins in seconds	
1	Pocket experiment.....	65	Positive 9	10	7	74
2	Pocket experiment.....	60	Positive 13	8	10	60
3	Ligated and cut both sympathetic trunks in thorax.....					
4-6	Pocket experiment with stimulation of both sympathetics 5 secs. on and 5 off, for 90 secs.....	90	Positive 12.4	10	15	42
8-10	Pocket experiment with stimulation of both sympathetics 5 secs. on and 5 off, for 90 secs.....	90	Positive 11	12	14	42
12	Divided both major splanchnics in abdo- men.....					
13	Pocket experiment.....	100	No	No		34
14-17	Pocket experiment with stimulation of sympathetics in tho- rax for 1 min.....	80	No	No		34
26-29	Pocket experiment with stimulation of right major splanchnic in abdomen for 2 min.....	150	Positive 18.2	3-4*	14	26
30	Pocket experiment.....	120	No	No		20

Left adrenal weighed 0.204 gram and contained 0.17 mgm. epinephrin.

Right adrenal weighed 0.216 gram and contained 0.18 mgm. epinephrin.

Blood pressure has been very low throughout and at the end of the experiment was 30 mm. The pocket was allowed to fill for estimation of the quantity of blood for 3 minutes. It contained 0.45 grams blood.

\* This rise although small was perfectly definite.

*Note.*—As with the low blood pressures in this experiment a definite preliminary cusp was not seen on the blood pressure curve at the point corresponding to the beginning of the epinephrin effect, the time of commencement of the blood pressure reaction given in the table is always at the beginning of the of rise.



TABLE 11

*Condensed protocol of experiment on cat 116. Weight, 2.76 kg. The animal is pregnant. The left superior cervical ganglion was excised 20 days before the experiment. Urethane, 4 grams. 2.00 to 3.20 p.m. pocket prepared. Cannul in carotid connected for blood pressure tracing. Cannula in external jugular vein and in trachea. Coeliac and mesenteric arteries not tied until later*

NO. OF OBSERVATION	TIME		DURATION OF POCKET IN SECONDS	PUPIL DILATATION IN SECONDS	BLOOD PRESSURE RISE		INITIAL BLOOD PRESSURE
					Milli-meters	Begins in seconds	
2	3.25	Pocket experiment.....	90	Positive 12.6	8	7	42
3		Pocket experiment.....	90	Positive 12.6	8	7	42
4		0.3 cc. adrenalin (1:150,000) injected..			4		42
5		0.6 cc. adrenalin (1:150,000) injected..			10		42
6		0.5 cc. adrenalin (1:150,000) injected..			8		42
7		0.4 cc. adrenalin (1:150,000) injected..			6		42
	4.00	Both major splanchnics divided in abdomen.....					
9	4.05	Pocket experiment.....	90	No	No		34
		Clamped abdominal aorta and coeliac and mesenteric arteries...					
10		Pocket experiment.....	90	No	No		62
11		Pocket experiment.....	90	No	No		70
12		Pocket experiment with stimulation of right splanchnic (off and on).....	90	Very good 13.6	25	9	60
13		Pocket experiment with stimulation of right splanchnic (off and on).....	90	Very good 13.8	25*	11	60
18		0.3 cc. adrenalin (1:150,000) injected..			27		50
19		0.2 cc. adrenalin (1:150,000) injected..			20		50
20	4.55	Pocket experiment with stimulation of right splanchnic (5 secs. on, 5 secs. off).....	150	Very good 12.8	31	10	40

TABLE 11—Continued

NO. OF OBSERVATIONS	TIME		DURATION OF POCKET IN SECONDS	PUPIL DILATATION IN SECONDS	BLOOD PRESSURE RISE		INITIAL BLOOD PRESSURE
					Milli-meters	Begins in seconds	
21		P o c k e t experiment with stimulation of right splanchnic (5 secs. on, 5 secs. off)...	90	Positive 16	17	11	42
22		P o c k e t experiment with stimulation of right splanchnic (5 secs. on, 5 secs. off)...	135	Positive 16	12	11	42

Left adrenal weighed 0.185 gm. and contained 0.11 mgm. epinephrin.

Right adrenal weighed 0.200 gram and contained 0.09 mgm. epinephrin.

The smaller rise of pressure in observation 22 as compared with 20 and 21 indicate temporary exhaustion.

The blood pressure at the end was 48 mm. The pocket was allowed to fill for 2 min. The quantity of blood in it was 1.01 gm.

\* Curve was practically an exact replica of that obtained in observation 12, and shown in figure 3.

widening of palpebral fissure) on release. The amount of epinephrin liberated during the stimulation was assayed by adrenalin observations, such as 18 and 19, at 0.0005 mgm. per minute per kilo of animal. In comparing this amount with that spontaneously liberated at observation 2, it must be remembered that only one splanchnic was stimulated artificially, and that we have therefore only the output of one adrenal. Further, the splanchnic stimulation did not last for the whole period of closure of the pocket and the nerve was really only stimulated for half the nominal time of excitation (5 seconds stimulation at a time, always succeeded by an interval of 5 seconds rest). These observations, then, form no exception to the general rule, that more epinephrin is given off during artificial stimulation of the splanchnic than is spontaneously liberated in the same time. Although this fact would indicate that it might be easier to demonstrate the liberation of epinephrin by artificial stimulation of the sympathetics in the thorax after section of the major splanchnic, than its spontaneous liberation, such experiments

as we have made with stimulation of the sympathetic have not yielded positive results. This may be because of the deterioration of the condition of the animal, indicated by a definite

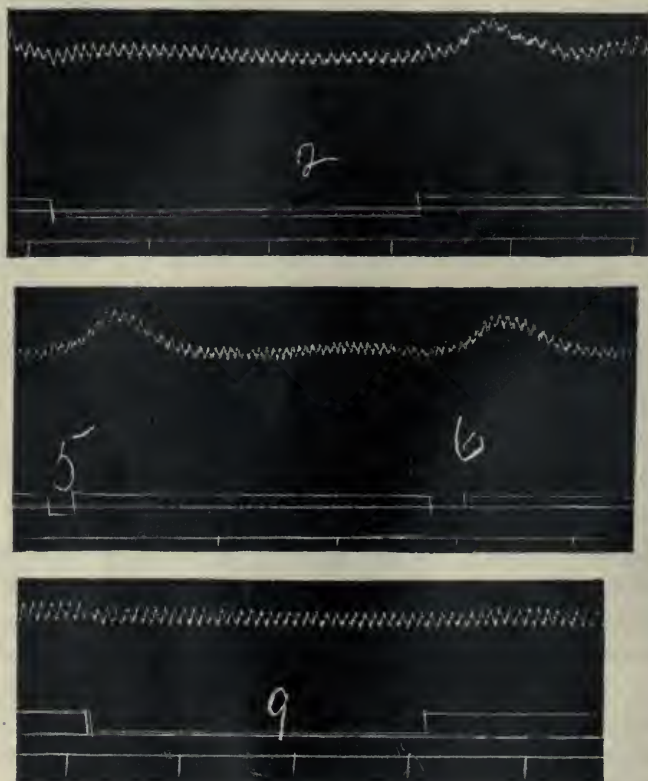


FIG. 14. CAT 116.

2, pocket experiment showing epinephrin rise after releasing the pocket (spontaneous liberation). 5, injection of 0.6 cc. adrenalin (1:150,000). 6, 0.5 cc. of same solution. 9, pocket experiment after section of major splanchnics in abdomen. No evidence of epinephrin liberation. The line of zero pressure is just below upper signal line.

drop in the blood pressure, when, in addition to the rather extensive operation in the abdomen entailed by the making of the cava pocket, the thorax is opened for isolation of the sympathetics. It is, in any case, not at all difficult to understand

that the comparatively slow processes which, in Elliott's experiments on cats and in ours on dogs, are associated with exhaustion of the epinephrin store in an adrenal whose innervation is intact, should produce in 6 or 7 hours very distinct changes, whereas an artificial stimulation lasting for two or three minutes although producing an effect, might not cause a detectable one.

#### EFFECT OF ASPHYXIA AND STIMULATION OF SENSORY NERVES UPON THE LIBERATION OF EPINEPHRIN

It has been shown by Elliott (5) that stimulation of sensory nerves causes diminution of the epinephrin store of the adrenals. Cannon and Hoskins (7) have stated that stimulation of sensory nerves and asphyxia produce so great a liberation of epinephrin into the blood that blood collected from the inferior cava by a catheter, passed into it from the femoral vein, gives with intestinal segments marked epinephrin reactions. We have made some experiments by the cava pocket method to test the question of liberation of epinephrin into the blood during electrical stimulation of afferent nerves and during asphyxia. The central end of the brachial nerve was used for stimulation, since with the permanent pocket the sciatic is not available. Asphyxia was produced by closing the tracheal cannula for longer or shorter periods while blood from the adrenals was being collected in the pocket. The asphyxia was stopped some time before the opening of the pocket in order to allow the blood pressure curve, the respiratory variations in which were of course enormously increased during the asphyxia, to become more nearly normal. In some observations the tracheal cannula was not closed until the pocket had been clipped off. In others asphyxia preceded the closing off of the pocket and lasted for a certain time during its closure. The idea was that if asphyxia was producing an increased liberation of epinephrin, and the effect began immediately, the former set of observations would enable blood with a maximum content of epinephrin to be collected, whereas if the asphyxia did not cause its maximum effect until a little time had elapsed, blood richer than normal in epinephrin would



still be caught in the pocket in the latter set of observations. Nevertheless, by neither modification of the experiment have we been able to find any definite increase in the epinephrin liberated during asphyxia as compared with that liberated in control observations in which the animal was breathing normally. If there is any increase at all in such relatively short periods of asphyxia as can be employed (up to about 2 minutes) it is too small to give an unequivocal difference by the methods we have used. With stimulation of the brachial nerves we have not obtained any increase whatever. The experiments are illustrated by a condensed protocol of one of them given in table 12, and by specimens of the tracings reproduced in figures 15 to 18.

It ought in fairness to be stated that the experiments on stimulation of afferent nerves can be done more exactly than those on asphyxia. For first, the blood pressure curve, with the restricted circulation entailed by the making of a permanent cava pocket, is not greatly affected by stimulation of the brachial, and whatever effect is produced ceases practically with the stoppage of stimulation, so that any epinephrin rise after the release of the pocket is easily detected. Secondly, the eye reactions, if present before, are still available after brachial stimulation. On the other hand, asphyxia causes great distortion of the blood pressure curve and also dilatation of both pupils, so that the pupil reaction of the denervated eye cannot be so easily studied. It may further be pointed out that if a slightly greater epinephrin reaction may sometimes appear to be obtained in an asphyxia observation than in the control, the more rapid filling of the pocket with blood due to the increased arterial pressure during the asphyxia might account for the difference.

The experiments on stimulation of the brachial cannot be compared with Elliott's observations on the effect of prolonged afferent stimulation (several hours) in causing exhaustion of the epinephrin store of the adrenals. In any case, there is no reason to suppose that conditions which diminish the stock of epinephrin in the adrenals must necessarily increase the rate of liberation of that substance into the adrenal veins. The diminu-

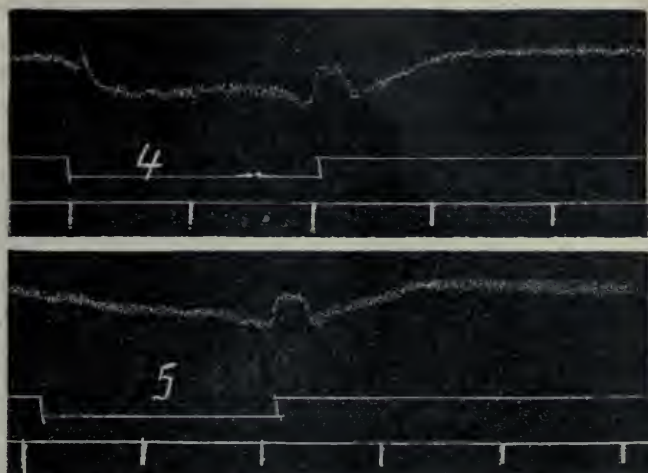


FIG. 15. CAT 95

4, Pocket with brachial stimulation. 5, control pocket without brachial stimulation. Line of zero pressure moved up towards the curve 55 mm.

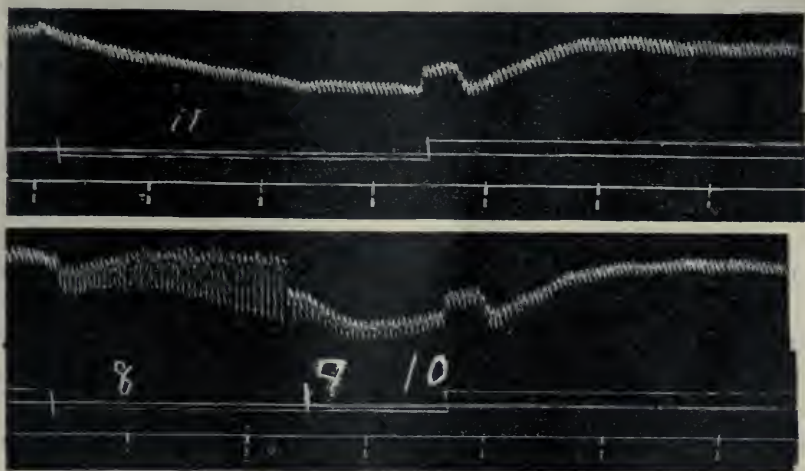


FIG. 16. CAT 95

8, Pocket with asphyxia. Asphyxia stopped at 9, pocket opened at 10. 11, control pocket without asphyxia. Line of zero pressure moved up 50 mm.

tion in the stock may be due to interference with formation of the substance. On the other hand, our observations ought to be capable of comparison with those of Cannon and Hoskins on the liberation of epinephrin into the blood since they also used short periods of stimulation of sensory nerves and of asphyxia. The difference between their results and ours is puzzling. It cannot depend upon the greater sensitiveness of the method

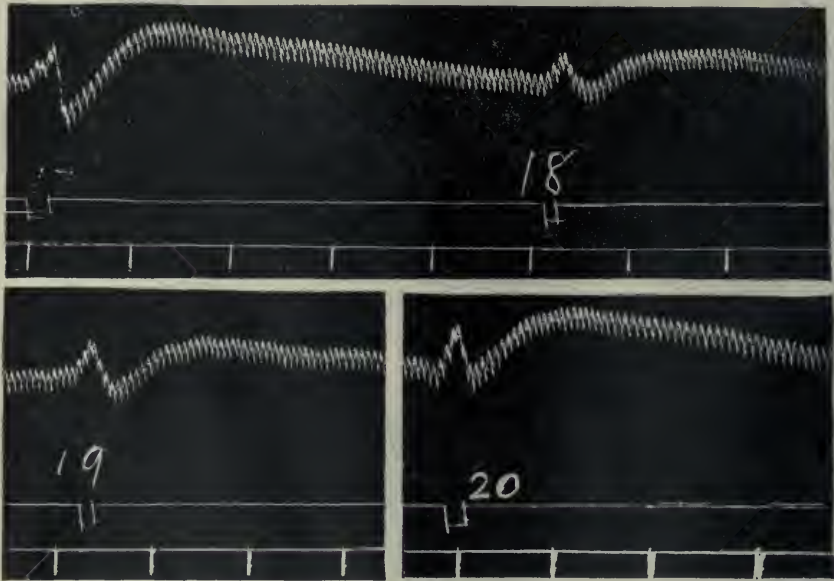


FIG. 17. CAT 95

Adrenalin assay. 17, 0.8 cc. of 1:125,000 adrenalin injected. 18, 0.4 cc.; 19, 0.5 cc.; 20, 0.6 cc. of the same solution. Line of zero pressure moved up 45 mm.

adopted by them (the rabbit's intestine segment method first employed by one of us (S) (8), and by Hoskins (9)). For we obtain positive epinephrin reactions from cava pocket blood collected without asphyxia or electrical stimulation of afferent nerves, whereas Cannon and Hoskins state that cava blood taken by the catheter without asphyxia or sensory stimulation caused no inhibition of the intestinal segments, although blood collected



from the catheter during asphyxia and sensory stimulation caused marked inhibition of the segments. It is, of course, possible that with the more extensive operation in our observations the spontaneous discharge of epinephrin is already so much increased that there is no room for a detectable increase by as-

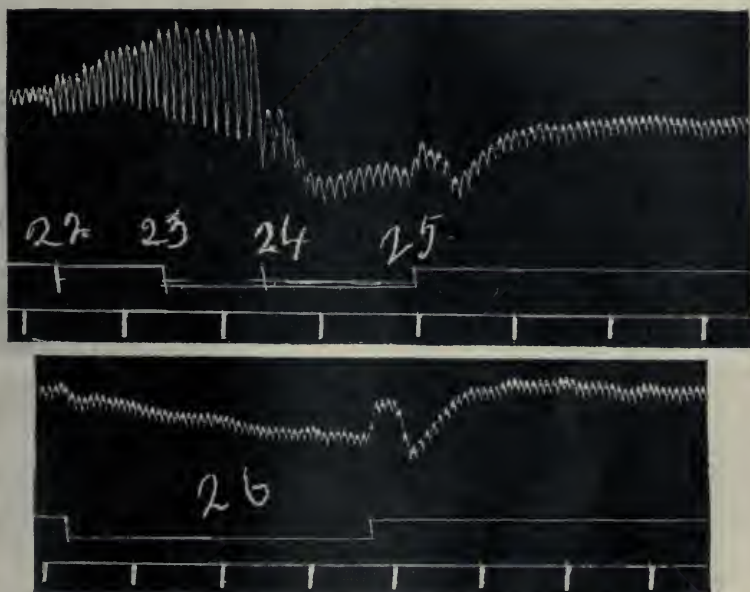


FIG. 18. CAT 95

Asphyxia begun at 22; stopped at 24. Pocket closed at 23, opened at 25 26, control pocket experiment without asphyxia. Line of zero pressure moved up 45 mm.

phyxia, etc. Tschoboksaroff (1) concluded that "the increase of blood pressure caused by stimulation of a sensory nerve (sciatic) has no effect on the quantity of secreted adrenalin." In his experiments also the operative procedure was more severe than in those of Cannon and Hoskins.



TABLE 12

*Condensed protocol of experiment on cat 95. Weight, 3.275 kg. Left superior cervical ganglion excised 39 days before the experiment. Urethane, 4 grams. Permanent cava pocket prepared with ligation of arteries. Both vagi divided. Cannula in carotid, external jugular and trachea.*

NO. OF OBSERVATION		DURATION OF POCKET IN SECONDS	PUPIL DILATATION IN SECONDS	BLOOD PRESSURE RISE		INITIAL BLOOD PRESSURE
				Milli-meters	Begins in seconds	
1	Pocket experiment.....	50	Positive 7	10	7	150
2	Pocket experiment.....	55	Positive 6	12	6	146
3	Pocket experiment with stimulation of brachial..	62	Positive 7.6	12	7	136
4	Pocket experiment with stimulation of brachial...	64	Positive 6.4	12	6	132
5	Pocket experiment.....	60	Positive 6.6	12	7	138
6	Gave ether.....					
7	Pocket experiment with asphyxia.....	60	Positive 11	*		
8-10	Pocket experiment with asphyxia for 1 minute....	100	Positive 6.2	13	8	140
11	Pocket experiment.....	100	Positive 6.4	11	8	140
17	0.8 cc. adrenalin (1:125,000).....			23		130
18	0.4 cc. adrenalin (1:125,000).....			8		128
19	0.5 cc. adrenalin (1:125,000).....			12		130
20	0.6 cc. adrenalin (1:125,000).....			17		132
22-25	Pocket experiment with asphyxia begun before closing pocket and lasting 1 minute.....	105	Positive 7.2	20	8	140
26	Pocket experiment.....	105	Positive 9.2	20	9	130
	Cut both major splanchnics in abdomen.....					
27	Pocket experiment.....	90	Too wide	6	15	90
28-31	Pocket experiment with asphyxia.....	90	Both dilated.	7	15	100
32-34	Pocket experiment with brachial stimulation.....	120	Slight 17.6	9	12	108
38	Pocket experiment.....	100	Slight 14	10	20†	114

\* The rise present was disturbed by the spontaneous recovery of the blood pressure from the ether depression so that the amount of the rise could not be determined.

† The beginning of the small fall of pressure which precedes the rise in the epinephrin reaction is what is given in this column in all observations, except 38, in which the cusp was absent.

TABLE 12—Continued

NO. OF OBSER- VATION		DURATION OF POCKET IN SECONDS	PUPIL DILATATION IN SECONDS	BLOOD PRES- SURE RISE		INITIAL BLOOD PRESSURE
				Milli- meters	Begins in seconds	
39	Pocket experiment.....	120	Fair 12.6	16	13	114
	Both semilunar ganglia freed from strands com- ing to them					
40	Pocket experiment.....	120	No	No		68
41-44	Pocket experiment with stimulation of brachial	165	No	No		70
	Now allowed pocket to fill for 2 min., 50 secs. Quantity of blood in pocket 1.32 grams.					

Left adrenal weighed 0.200 gram and contained 0.17 mgm. epinephrin.

Right adrenal weighed 0.208 gram and contained 0.18 mgm. epinephrin.

## SUMMARY

1. The spontaneous liberation of epinephrin has been studied (in the cat) by means of the (denervated) eye reactions and the blood pressure changes caused by blood from the adrenals when permitted to pass into the circulation from a pocket of the vena cava in which it has been collected in known amounts and for known periods of time.

2. Since the blood is not withdrawn from the vessels the uncertainty introduced by the rapid development in the blood of pressor bodies which simulate the action of epinephrin on some of the objects most generally used in biological tests for that substance, is eliminated.

3. The simultaneous observation of the eye reactions greatly aids in the interpretation of the blood pressure curves when the amount of epinephrin is small.

4. The approximate assay (without withdrawal of blood) of the epinephrin in the blood collected in the cava pocket from the adrenals, by the injection of varying doses of adrenalin generally presents no difficulty. It must be repeated from time to time in the course of an experiment when the condition of

the animal changes. The amount of epinephrin spontaneously liberated in cats was found to vary in different experiments within a rather narrow range considering the differences in the conditions (from 0.0008 to 0.0028 mgm. per minute per animal, or from 0.0003 to 0.001 mgm. per minute per kilo of animal).

5. After section of both sympathetic trunks in the thorax near the diaphragm, including the major splanchnics, the spontaneous liberation of epinephrin is completely abolished. Division of the major splanchnics in the abdomen does not necessarily cause total cessation of the secretion in all cats. In one animal a detectable amount was still liberated but the liberation was entirely stopped when all the fibers coming to the semilunar ganglion were cut.

6. The fall of blood pressure caused by section of both splanchnics has nothing to do with the failure of the adrenals to liberate epinephrin. For when the nerves of the right gland are alone divided and the left adrenal vein clipped, the blood collected from the right adrenal in the cava pocket yields no epinephrin reactions on release of the pocket.

7. Although, as is known, cats survive indefinitely the removal of one adrenal and division of the nerve supply of the other, no detectable epinephrin was found in the blood coming from the remaining adrenal 5 weeks after the operation. Good reactions were obtained on massaging the gland.

8. No increase in the epinephrin liberation was detectable when sensory nerves (brachial) were stimulated. If any increase was produced by asphyxia in our observations it was very slight.

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## THE INFLUENCE OF THE ADRENALS ON THE KIDNEYS<sup>1</sup>

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In the course of an investigation (1) on the distribution of urea in body fluids and tissues, we had occasion to analyze the tissues from two dogs which had died as the result of double adrenal extirpation. These showed about five times the normal content of urea. This rather suggested that after the removal of the adrenal glands from animals, there exists either a condition of renal insufficiency or a greatly increased protein catabolism. The present investigation was undertaken to determine whether an accumulation of nitrogenous products in the blood and tissues was a constant condition after complete removal of the adrenal glands, whether increased protein catabolism or renal insufficiency or both were essential to explain these changes, and whether or not an interrelationship between the adrenals and kidneys existed.

Our earlier work confirmed these first observations that animals deprived of both adrenals died with an increased non-protein nitrogen and urea content of the blood. However, all the animals of this series, including several cats and a few dogs, lived only about twenty-four hours after the excision of the glands. Consequently, the findings of the accumulation of nitrogenous products in the organism might easily be explained as a terminal event dependent upon the moribund condition of

<sup>1</sup> Presented in abstract before the American Society for Pharmacology and Experimental Therapeutics, Boston, December 27, 1915. See Proceedings, this Journal, viii, p. 111, 1916.



the animals with low blood pressure and a general decrease of all bodily functions.

At this time, two communications bearing upon the problem came to our notice. Porak and Chabanier (2) reported finding an increased urea content of the blood in seven rabbits 8 to 12 hours after extirpation of the suprarenal capsules. On account of the short life of their animals, eight to twelve hours after the operation, their findings of increased blood urea may be simply, as has been suggested above, a terminal event. Another paper by Elliott (3) describing several years' experience with the excision of adrenal glands in cats demonstrated that by a removal of the two glands at an interval of three weeks or longer, and with certain other precautions, the life of the animals could be considerably lengthened. Moreover in these animals, the final muscular exhaustion and period of low blood pressure did not supervene until a day or so before death. In the meantime their condition was substantially normal, and could in no sense be called moribund. Our subsequent experiments were conducted exclusively upon cats with separate removal of the glands, and the studies were made before the muscular weakness occurred.

#### METHODS

In operating, the adrenal was removed through a lumbar incision, starting near the costal margin and running generally parallel to the fibers of the transversalis muscle. The lumbar vein was tied and cut on both sides of the gland, the gland loosened up by blunt dissection aided by a traction suture placed through it, and finally snipped out, close to the capsule, with scissors. The peritoneum was then closed over the adrenal site by a running or purse-string suture of fine silk. This procedure always arrested any slight oozing of blood, and prevented adhesions. The right adrenal was always removed first. Closure was performed in two layers, with obliteration of dead spaces. Aseptic precautions were observed and there were no infections. The animals were anaesthetized with ether given by the intra-tracheal method.

In procuring blood and urine from these cats, the customary proceedings were as follows: Blood was drawn from the jugular vein with pipette and needle, through the skin and without anaesthesia. Phenolsulphonaphthalein was given intramuscularly. Urine was collected under toluol in small metabolism cages, the bladder being emptied by catheterization at the end of each stated period. Since it is difficult to be sure that one has expressed all the urine from the cat's bladder, it was washed each time with distilled water until the irrigation returned colorless through the catheter. Measurement of this return made sure that no important part of the irrigation fluid remained in the bladder. This necessitated the use of female cats in practically all of the experiments. Blood pressure was measured in a few cases by placing a cannula directly in the femoral artery, under local anaesthesia. As a rule this caused no struggling. In such cases arterial blood was collected for the determinations.

Of the animals used, sixteen were operated on as above, both adrenals being removed at intervals of three to twelve weeks, usually about three to five weeks, and the duration of survival noted. Complete ablation of adrenal tissue was determined at autopsy, microscopic sections of the site of operation being made.

The accompanying table gives the duration of life after removal of both adrenals.

TABLE 1

Days.....	7	·6	5	3½	3	2½	2	1½	1	Average, 3½
Number of cats.....	3	1	1	1	1	1	6	1	1	Total, 16

The condition of the cats was excellent after the second operation. Sometimes they appeared quite normal, while in other cases there was a little languor. When animals in apparently good condition were tested, the blood pressure was found normal. As a rule, however, they did not eat or drink freely. The onset of serious symptoms was often comparatively sudden, occurring from 24 hours to a few minutes before death. These symptoms consisted of great muscular weakness and fall in blood pressure without loss of consciousness. About one-half hour

before death, coma and then convulsions usually appeared as noted by Albanese. Anything which excited the cat or caused it any exertion had a very deleterious effect, and indeed in some cases undoubtedly caused the onset of the serious ante-mortem symptoms. For example, one apparently healthy cat was seized with convulsions during catheterization, and died twenty minutes later. This fact is of significance in comparing the survival periods of our cats with those of Elliott's animals. In each case our cats were subjected to daily manipulations, as described for collection of urine or blood.

In addition to the principal series above described, the left splanchnic nerve was sectioned just above the diaphragm in two cats from which the right adrenal had been removed previously. These cats remained in good condition and showed no abnormal symptoms whatever.

The methods used in investigating the condition of the kidneys in adrenalectomized animals have been directed along four lines.

1. Renal function was investigated in adrenalectomized animals which survived for several days and could therefore be studied before the final muscular exhaustion and low blood pressure developed. The concentration of urea in the blood, and the excretion of phenolsulphonephthalein have been used in this connection.

2. An examination of nitrogenous metabolism during starvation was made before and after the removal of the adrenal glands.

3. The response of the kidneys in normal and adrenalectomized cats to an intravenous injection of a mixture of urea, creatinine and sodium chloride was determined. These substances were chosen as they are not changed in passing through the organism, are eliminated at different rates, and probably by a different mechanism on the part of the kidneys.

4. Specimens of the kidneys of operated animals and of normal animals were studied histologically. Control studies were made in several ways, and are indicated in their proper position in the experimental data which follow.



## RESULTS

*Urea retention.* The results of the functional tests are best illustrated by means of protocols and tables representing typical animals, which are given at the end of this section and the one following. It will be seen that, in all the cases, the blood urea shows a striking rise immediately following the second operation, reaching a level roughly twice as high as that existing previously. After this, no further change occurs until about twenty-four hours before death, when a second sharp rise presages a rapidly fatal issue. In animals surviving a shorter time, this plateau phenomenon does not become apparent, the urea retention increasing practically steadily until death. The retention occurred to substantially the same extent in every animal successfully operated upon with a single exception. In every case when a sufficient number of determinations was made, and where survival was long enough, the curve showed the plateau form. The exception noted (B20) showed a blood urea of 90 mgm. per 100 cc. two days after operation, after which the figure fell to a normal level (40 mgm.) where it remained until death, which was apparently a typical adrenal death. Concerning this animal, we can only suggest that the presence of an accessory adrenal might explain the findings. The large numbers of positives allow us to think that this one exception has little significance.

Donath (4) has stated that in adrenalectomized cats the total blood solids are increased from an average of 19.6 per cent to an average of 24.5 per cent. Such an increase in concentration of the blood would not alter the urea concentration, urea being equally distributed in plasma, corpuscles and tissues, unless a dehydration of the entire organism to a like extent had occurred. Even in this unlikely event the urea concentration would only be increased in the neighborhood of 10 mgm. per 100 cc. In addition, a cat in our series which was caused to thirst for twenty-five hours, showed absolutely no change in blood urea concentration.

Controls showed that animals not operated on, animals under-



going a simple ordinary operation, as cystotomy, and animals from whom only one adrenal was removed, showed no urea retention. A series of 28 determinations in normal cats showed blood urea figures ranging from 29 to 55 mgm. per 100 cc., the majority lying between 30 and 45 mgm. per 100 cc.

*I. Double adrenal extirpation, spontaneous death, without infusion.  
Cat B5*

*February 13, 1915.* Operation 10 a.m. Right adrenal removed.

*March 6.* Operation 10.30 a.m. Left adrenal removed. It is not hypertrophied. Animal on warm pad during operation. Recovers well and quickly from anaesthetic. Phthalein given just before operation, collection afterwards (two hours).

*March 7.* Condition excellent.

*March 8.* Condition good, though not showing quite as much spontaneous energy as before operation.

*March 9.* Refuses milk and water.

*March 10.* Condition unchanged.

*March 11.* Condition still good, will walk, but prefers to lie down in quiet corner.

*March 12.* 10.30 a.m. Very quiet today. When put on floor makes effort to walk, but staggers badly, soon lies down on abdomen. 11.30 a.m. Begins to cry, soon lies on side in cage, breathing slowly,

TABLE 2

Cat B5

DATE	PHTHALEIN	BLOOD UREA
	<i>per cent</i>	<i>mgm. per 100 cc.</i>
<i>First operation</i>		
February 13, 1915.....		
March 5.....	68	
March 6.....	63	
<i>Second operation</i>		
March 7, 1915.....	55	58*
March 8.....	35+	88
March 9.....	64	82
March 11.....	44	108
March 12.....		144
	death	

\* Blood taken just at end of operation.

deeply and irregularly. Does not respond to stimulation. Dies quietly about noon. Autopsy at once. Blood obtained from heart, which is still beating very feebly.

No adrenal tissue is found at sites of operation. Lungs show some congestion. Liver lobules show bright yellow central areas. Kidneys appear normal in gross, and also on subsequent histological examination.

*II. Double adrenalectomy (second transplanted before removal); spontaneous death; without infusion. Cat B9 (male)*

*February 25, 1915.* Operation 10 a.m. Right adrenal removed.

*March 8.* Operation 11 a.m. Left adrenal loosened by means of semilunar incision in peritoneum below, lumbar vein cut medially, gland displaced through operation incision to position beneath skin, where it is anchored. Connections with coeliac ganglion not severed. Recovery good.

*March 24.* Condition excellent. Left adrenal quickly removed under local anesthesia. No visible shock or other effect from this procedure.

*March 25.* No change.

*March 26.* Cat seems very well, but is disposed to lie down in cage when left alone, whereas previously it constantly moved about. When taken up, however, behaves as normal cat.

*March 30.* Condition still excellent. Blood pressure taken from femoral, 115 millimeters Hg.

*March 31.* Found in cage in morning, very weak and limp, legs twitching. Urinates freely just before death. Autopsy at once.

Ten cubic centimeters of normal cat's blood, and then 10 cubic centimeters of blood from B9 are injected intravenously into a normal cat connected to a blood-pressure manometer. No effect of any kind noted in either case.

Blood urea March 27, 60 milligrams per 100 cc., at autopsy 178 milligrams per 100 cc.

*Phenolsulphonophthalein excretion.* The experiments disclosed a tendency for the excretion of phenolsulphonophthalein to decrease to a slight extent following the second operation and more markedly as the premortal failure came on. The changes were not however as striking or as constant as those in the blood urea. In some cases the percentage observed after operation

was substantially the same as that before, in spite of the fact that during this period the blood urea remained constant at a high level. Some of the results are shown in tables at the end of this and the preceding sections.

It should be stated that the phenolsulphonephthalein test, as used by us in cats is not highly accurate, for a number of reasons. Intramuscular injections were made, and since the subcutaneous tissue is so loose, and the muscles so small, it is not always possible to be sure that all of the dye has gone into the muscles. The loss of a few drops of urine at the time of catheterization may make noteworthy differences, since the quantity is small and the concentration therefore high. Lastly, a considerable number of controls, in apparently normal cats showing no urea retention and kidneys histologically normal, gave results with wide variations, the average figure lying lower than that usually observed in dogs, rabbits and human beings. In a normal cat, two intramuscular injections gave respectively 45, 48.5 per cent excreted for two hours and one intravenous injection gave 60 per cent excreted. Other normals were 50, 54, 55, 64, 63, 69, 54 per cent. The same animals used in controlling urea retention were injected with phthalein, the results showing that neither simple operations, single adrenalectomies or etherization causes any fall in the output. In some cases the phthalein was injected just before beginning the operation and the output actually during operation and anaesthesia was shown to be normal.

*Metabolism.* The nitrogen excretion was studied as total urinary nitrogen<sup>2</sup> during starvation, but with water either given ad libitum or injected in definite quantities, as 0.85 per cent salt solution, under the skin daily. It showed in most cases a definite fall following the second operation, with another secondary and more pronounced fall in the premortal period. This result was not quite as striking and constant as the urea retention in the blood, but in general ran parallel to it, and

<sup>2</sup> No determinations of nitrogen in the feces were made, but during starvation this is too small in cats to affect the results. None of the animals exhibited diarrhoea.



accounted for the nitrogen concerned in the retention. This makes it unnecessary to imagine any increased nitrogenous metabolism in these animals. Typical tables are shown herewith.

In the animals living longest (B20, B29) nitrogen excretion appeared to improve a little towards the end of the period of level blood urea. This phenomenon would necessarily occur, with the blood urea remaining level, unless nitrogenous catabolism were definitely decreased.

The conditions under which the functional and metabolic studies were made were the same, since they were all usually carried out on the same animals. In most of them, starvation was instituted forty-eight hours before the second operation, and continued until death. In the few where food was given, conditions were practically the same, since they uniformly refused food.

As a rule, water was also poorly taken. Consequently in a number of cases subcutaneous infusions of salt solution were given, about 50 cc. per day, beginning either simultaneously with the starvation, or immediately following the second operation. In these animals no other fluid was allowed. Our series is not long enough to permit us to say whether this procedure lengthens the life of adrenalectomized animals, as it did in the experience of Soddu (5). The quantity of urine was greater, but there was no fundamental alteration in the nitrogen excretion in the urine or retention in the body.

Controls which were made similarly to those previously described with simple operations and single adrenalectomies showed that none of these procedures, or the attendant etherization, produced any decrease in the urinary nitrogen output. The control animals here referred to were the same as those used in controlling the blood urea and phenolsulphonephthalein studies.

*III. Double adrenalectomy; spontaneous death; salt solution infusion before and after second operation. (Also as control, during first operation). Cat B25*

*May 4, 1915.* Started on starvation.

*May 6.* Operation 3.30 p.m. Right adrenal removed.

*May 9.* Put back on routine feeding.



May 28. Started on starvation.

May 29. Received 50 cubic centimeters 0.85 per cent NaCl solution subcutaneously every day from now on.

May 31. Operation 2.15 p.m. Left adrenal removed. Moderately hypertrophied.

June 1. Cat seems rather weak and flabby.

June 2. Dies 11 a.m., as blood is being drawn.

Autopsy. Organs, including kidneys, appear normal. No adrenal tissue found, in gross or microscopically.

TABLE 3

B25

DATE	PHTHALEIN	BLOOD UREA	URINARY N
	<i>per cent</i>	<i>mgm. per 100 cc.</i>	<i>grams</i>
May 5, 1915.....	52		2.22
May 6.....	70	47	1.84
<i>First operation</i>			
May 7.....		51	1.67
May 8.....	68		1.83
May 28.....			0.74
May 30.....			0.92
May 31.....		31	0.80
<i>Second operation</i>			
June 1.....	24		0.44
June 2.....		144	0.23
	death		

#### IV. Double adrenal extirpation, spontaneous death, daily salt solution infusions. Cat B29

October 29, 1915. Operation 4 p.m. Right adrenal completely removed.

November 16. Put on starvation, catheterized daily for urinary nitrogen.

November 17. Fifty cubic centimeters 0.85 per cent NaCl solution subcutaneously daily from now on.

November 20. Operation 10 a.m. Left adrenal completely removed. On warm pad during operation. Recovers slowly but well from anaesthetic.

November 21. Animal in good condition, though does not show as much spontaneous energy as before operation.

*November 22.* Animal somewhat weak today, but can still walk with some swaying.

*November 23.* Condition the same as yesterday.

*November 25.* Condition still much the same. Cat now apathetic. Bladder sphincter has lost some of its tone.

*November 26.* Condition the same.

*November 27.* Cat dies at 9 a.m. Last symptoms not noted. Blood collected from heart.

*Autopsy.* No adrenal tissue found at adrenal sites. Other organs appear normal. Kidneys appear normal in gross and microscopically.

TABLE 4

B29

DATE	PHTHALEIN	BLOOD UREA	URINARY N	UREA PER CENT OF TOTAL NITROGEN
	<i>per cent</i>	<i>mgm. per 100 cc.</i>	<i>grams</i>	
November 18, 1915.....	45		1.16	83
November 19.....				
November 20.....		38	1.06	84
<i>Second operation</i>				
November 21.....			0.70	71
November 22.....	24	95	0.93	82
November 23.....	30		0.66	82
November 24.....		92	0.94	
November 25.....	10		0.86	
November 26.....		105	1.34	
November 27.....		165	0.23	

*V. Double adrenalectomy; spontaneous death; without infusion. Cat B30*

*October 30, 1915.* Operation 11 a.m. Right adrenal removed. Recovers well.

*November 16.* Starvation started.

*November 20.* Operation 2 p.m. Left adrenal removed.

*November 21.* Condition good, satisfactory muscular tone, though not as much spontaneous energy as before operation.

*November 22.* Condition not quite so good today, less muscular tone.

*November 23.* Found dead in the morning.

*Autopsy.* No adrenal tissue in the gross or microscopically. Kidneys appear normal in gross and microscopically.

TABLE 5  
B30

DATE	PTHALEIN	BLOOD UREA	URINARY N	UREA PER CENT OF TOTAL NITROGEN
	<i>per cent</i>	<i>mgm. per 100 cc.</i>	<i>grams</i>	
November 18, 1915.....	41		0.79	82
November 19.....				
November 20.....		55	1.02	83
<i>Second operation</i>				
November 21.....			0.76	85
November 22.....	40	89	0.75	79
November 23.....		128 death	0.19	84

*Sections of splanchnics.* In the animals in which the splanchnic nerve was cut above the adrenal as described above, no urea retention occurred in the blood, and the animals remained to all appearances normal. Autopsy confirmed the section of the nerve.

*V. Splanchnotomy. Cat B53.*

*June 7, 1916.* Right adrenal completely removed.

*June 28.* Operation 1.30 p.m. Left splanchnic nerve cut just above diaphragm. Cat put on starvation.

*July 3.* Receives food again today.

*July 5.* Animal has remained in perfect condition. Sacrificed.

*Autopsy.* Operation site in perfect condition, no adhesions or exudate. Splanchnic cut between levels of first and second lumbar vertebrae, about 1 centimeter above coeliac ganglion. No branches join it from spinal cord below point of section. No trace of right adrenal found. Kidneys appear normal in gross. Other organs appear normal.

TABLE 6  
B53

DATE	PTHALEIN	BLOOD UREA
	<i>per cent</i>	<i>mgm. per 100 cc.</i>
June 28, 1916.....	50	41
<i>Splanchnotomy</i>		
July 1, 1916.....	61	41
July 3.....	61	
July 5.....		45

*Response of the kidneys to injection of urea, creatinine and sodium chloride.* The cats used in these experiments were divided into two series, the controls and the experimental animals. The former were normal and singly adrenalectomized animals, the latter doubly adrenalectomized. An injection of 15 cc. per kilo of a solution of 3.5 per cent each of urea and sodium chloride, and 0.2 per cent creatinine was given intravenously to each animal. The experiment was continued for two and one-half hours, the urine collected in three or five periods and analyzed for urea, creatinine and chlorides. The concentration of these three substances was determined in the blood before the injection and at the end of the experiment. As urea (6) and creatinine (7) are equally distributed in body fluids and tissues, the relative increase in concentration of these substances in the blood at the end of the experiment serves as a control of the amounts excreted in the urine.

This procedure is, of course, a test of the ability of the kidney to excrete the three substances. The increase over normal in the urine and the rate of excretion of urea, creatinine (8) and sodium chloride have been proposed as tests of renal function when definite quantities of one of these substances are given by mouth.

The experimental animals, having had one adrenal extirpated for two or three weeks, had the remaining gland removed about eighteen hours before beginning the observations, and for control purposes, other cats had one gland removed also eighteen hours before.

The animals were starved and given water ad libitum for forty-eight hours before the experiments. A twenty-four hour collection of urine was made ending on the morning of the experiment. The cat was catheterized and the bladder washed with warm water. A sample of blood was removed from the jugular vein for analysis. The injection of the urea—creatinine—chloride mixture was immediately made through a cannula inserted into the saphenous vein under infiltration anaesthesia with distilled water. The injection occupied about five minutes. At half-



hour or hour intervals the urine was collected by catheterization and the bladder washed with warm distilled water and the urine and the washings saved for analysis; at the end of two and one-half hours a sample of blood was drawn and in certain cases the blood pressure taken from the femoral artery under local anaesthesia by means of the apparatus described by Turner (9). The experiment was now discontinued. No anaesthetic was used during the experiment, and in the intervals between catheterizations the animals were removed from the table and placed in cages.

The specimens of blood and urine were analyzed for urea, creatinine and chlorides. Urea was determined by the urease method described by one of us, (10) creatinine by that of Folin (11) and chlorides by the procedure recently proposed by McLean and Van Slyke (12). The urine specimens for each period and bladder washings were analyzed separately. From a consideration of the percentage content of each of the three substances in the undiluted urine and washings respectively calculation of the amount of urine present in the washings was made. An average value of the amounts obtained by using the percentage of each substance was taken. The twenty-four hour specimen of urine collected before the experiment gave the daily normal excretion of urea, creatinine and chlorides for the animal. From this was calculated the amount of these three substances which would normally be eliminated by the cat in two and one-half hours. A comparison could now be made of the rate and amount of water, urea, creatinine and sodium chloride eliminated by the control and experimental animals. The blood analyses referred to above served as a check upon the deductions drawn from the urine, as a decreased elimination of any substance after injection would give rise to a greater difference in amount of that substance present before and at the end of the experiment in the blood.

The following tables are typical of the experiments.

TABLE 7

*Cat. B35, normal. Weight 2.96 kilos. Injection experiment December 4, 1915*

TIME AFTER INJECTION	URINE	AMOUNT INJECTED					
		1549 mgm.		88.5 mgm.		1549 mgm.	
		Urea		Creatinine		Chloride	
minutes	cc.	mgm.	per cent	mgm.	per cent	mgm.	per cent
30	9.1	254	2.79	30	0.33	66	0.73
60	7.9	237	3.00	17	0.22	90	1.14
90	7.2	256	3.56	14	0.19	103	1.52
150	14.9	451	3.03	21	0.14	259	1.74
Total.....	39.1	1198		82		534	
Corrected.....		1100		72			

TABLE 8

*Cat B54, July 13, 1916, right adrenal removed. Weight 2.24 kilos. Injection experiment July 14, 1916*

TIME AFTER INJECTION	URINE	AMOUNT INJECTED			
		1176 mgm.		67.2 mgm.	
		Urea		Creatinine	
minutes	cc.	mgm.	per cent	mgm.	per cent
30	14.8	290	1.96	26.6	0.18
90	15.1	476	3.15	30.2	0.20
150	11.0	351	3.19	17.6	0.16
Total.....	40.9	1117		74.4	
Corrected.....		867		63.0	

TABLE 9

*Cat B34. Right adrenal gland removed December 3, 1915. Second gland removed December 17, 1915. Injection made December 18, 1915. Weight 2.40 kilos.*

TIME AFTER INJECTION	URINE	AMOUNT INJECTED					
		1260 mgm.		72 mgm.		1260 mgm.	
		Urea		Creatinine		NaCl	
minutes	cc.	mgm.	per cent	mgm.	per cent	mgm.	per cent
30	7.4	111	1.50	16	0.21	68	0.92
60	8.2	132	1.58	12	0.15	86	1.05
90	7.1	119	1.68	9	0.13	76	1.07
120	7.5	119	1.59	8	0.12	72	0.96
150	4.5	86	1.90	6	0.13	46	1.02
Total.....	34.7	567		51		348	
Corrected.....		463		40			

TABLE 10

*Cat B48. Right adrenal gland removed on February 10, 1916. Second gland removed April 11, 1916. Injection made April 12, 1916. Weight 2.70 kilos*

TIME AFTER INJECTION	URINE	AMOUNT INJECTED			
		1417 mgm.		81 mgm.	
		Urea		Creatinine	
<i>minutes</i>	<i>cc.</i>	<i>mgm.</i>	<i>per cent</i>	<i>mgm.</i>	<i>per cent</i>
30	9.9	161	1.63	16	0.18
90	2.2	47	2.13	6	0.25
150	8.1	132	1.68	13	0.16
Total.....	20.2	340		35	
Corrected.....		259		25	

Table 11 gives the figures of the analyses of the blood for urea and creatinine before the injection and at the end of the experiment, two and one-half hours after the injection.

Table 12 gives a summary of the results on the excretion of urea and creatinine during the two and one-half hour period; as stated above and shown in typical protocols the excretion was measured every half-hour. As we found, no noticeable difference, however, in the rate of excretion in the case of control or experimental animals, the complete figures are not given. The urea percentages were in general much higher in the urine of the controls than of the operated animals; the creatinine showed a steadily decreasing percentage and the chloride a steadily increasing percentage in the controls, while in the experimental animals they remained more constant or varied without rule.

Owing to the wide variations found in normal animals in the excretion of sodium chloride after injection under the conditions of the experiments, definite conclusions in regard to the relative ability of the kidneys of the control and experimental animals to excrete chlorides can not be drawn. Since chlorides are very unevenly (13) distributed in body fluids and tissues and there apparently exists a threshold value for the blood below which no chloride is excreted, a comparison of the chloride content of

TABLE 11  
*Analyses of blood for urea and creatinine*

		NORMAL			ONE ADRENAL OUT		Average	BOTH ADRENALS OUT					
		B35	B36	B44	B54	B55		B34	B41	B42	B37	B48	Average
Urea mgm. per 100 cc.	Before injection...	48	45	43	42	36	43	61	78	61	81	50	66
	After experiment..	75	69	69	62	63	67	116	138	103	140	108	121
	Difference.....	27	24	24	20	27	24	55	60	42	59	58	55
Creatinine mgm. per 100 cc.	Before injection...	1.1	1.0	1.0	0.8	1.0	1.0	1.0	1.2	1.2	1.2	1.3	1.2
	After experiment..	1.4	1.1	1.7	0.9	1.2	1.2	2.9	2.5	2.4	3.0	3.8	2.9
	Difference.....	0.3	0.1	0.7	0.1	0.2	0.3	1.9	1.3	1.2	1.8	2.5	1.7
	Blood pressure at end of ex- periment.....				117	145*	125				113	80	85

\* This animal struggled.

TABLE 12  
*Excretion of urea and creatinine after injection*

		NORMAL		ONE ADRENAL OUT		Average	BOTH ADRENALS OUT					
		B35	B36	B54	B55		B34	B41	B42	B37	B48	Average
	Urine, cc.....	39.1	28.1	40.9	67.8		34.7	28.0	32.1	8.9	20.2	
Urea	Milligrams injected...	1549	1480	1179	1438		1260	1400	1400	1155	1400	
	Milligrams excreted..	1100	1167	867	922		463	440	629	151	259	
	Per cent excreted....	71	79	74	65	72	37	31	45	13	18	29
Creatinine	Milligrams injected..	88.5	84.6	67.2	82.2		72.0	80.0		66.0	80.0	
	Milligrams excreted..	72.0	79.0	63.0	69.2		40.0	39.0		28.0	25.0	
	Per cent excreted....	81	94	93	84	88	56	49		42	31	45

the blood before and after the experiment gives very little information. The following table gives the results obtained on chloride excretion.

*Histology.* The normal histology of the cat's kidney has certain peculiarities consisting mainly of the presence of considerable quantities of fat in the epithelial cells, and therefore a series of cats apparently normal were sacrificed for microscopic study



TABLE 13  
*Excretion of chlorides*

		NORMAL			BOTH ADRENALS OUT			
		B35	B44	B36	B34	B41	B42	B37
Plasma milligrams per 100cc.	Before injection...	700	656		728	723	816	800
	After experiment..	822	743		838	861	819	892
	Difference.....	122	87		110	138	3	92
Urine.....	Milligrams injected.....	1549	892	1480		1401	1400	1155
	Milligrams excreted.....	534	49	586		225	449	77

and comparison. It was also shown that the phenolsulphonophthalein excretion was normal. Herxheimer stains of frozen sections, as well as the ordinary hematoxylin and eosin preparations, were used. The fat content of the various kidneys was found to vary somewhat for unknown reasons. The animals had usually been on uniform diet for some time previous to the experiments. However it was not difficult to arrive at a satisfactory conception of the appearance of normality.

In those cats which had undergone double adrenal extirpation, and had then been allowed to survive as long as possible, no constant noteworthy alterations in the kidney picture were observed. In those which had been injected with the urea, creatinine and sodium chloride solution, however, there was a striking and characteristic picture. The cells of the convoluted tubules were so filled with large vacuoles that the remaining protoplasm consisted merely of a few threads forming a net work in the cell body, the nuclei had become small, irregular and pyknotic, and the lumina of the tubules was obliterated. The labyrinths therefore presented the appearance of an area of lacework. The Herxheimer stain applied to one of these typical kidneys showed that these vacuoles represented fat globules of all sizes, mostly quite large, so extensive that the labyrinths were transformed almost into solid red masses. In one animal of the series this change was less marked, but this animal died just at the end of the experiment.

This same change was visible to a much less degree in the kidneys of normal control cats which had also received the injections, and in those of the doubly operated cats which had received subcutaneous injections of salt solution. In these cases the change was wholly confined to a narrow zone at the outer edge of the cortex.

One of the cats showed a few adherent glomeruli on section. In this animal however, a small piece of adrenal was left in at operation, and the results from it have not been used.

In another case, a supposedly normal cat used for a control of the urea, creatinine and sodium chloride injection showed much diminished excretory powers. Autopsy revealed yellow firm kidneys with numerous depressed areas on the surface. Under the microscope, a spontaneous chronic nephritis of vascular type with fibrous and adherent glomeruli was seen.

Aside from these two instances, no chronic changes were seen in any of the kidneys.

#### DISCUSSION

The results of our operations confirm in general the experiences of Elliott and other previous workers on adrenal problems. Our animals have always succumbed after complete operations, with symptoms similar to those described in the literature.

The increased concentration of urea in the blood of cats after complete removal of the adrenal glands has been shown to be a constant phenomenon, and not necessarily a terminal event. In animals which survive for several days after the extirpation of the glands, the blood urea rises shortly after the operation and attains a concentration about double the normal value, at which it remains with slight fluctuations until about twenty-four hours before death, when a further steady rise occurs. To explain this phenomenon, one can imagine either a greatly increased protein catabolism or an impairment of kidney function. The experiments on the excretion of nitrogen in starving cats before and after double adrenalectomy have shown that no important change in the protein catabolism takes place. Mariani (14) and Gradinescu (15) claim that a decrease in the

ratio of urea N to total urinary N occurs after adrenal extirpation. Mariani gives no figures at all, while Gradinescu's figures do not invite confidence since in one case the urea N is given as greater than the total N, and in addition the method of urea determination (nitrogen estimation in phosphotungstic acid precipitate of urine) employed by him is grossly inaccurate.

We have made a few determinations of urea N percentages, and found that they do not appreciably vary before and after the removal of the adrenal glands. So one can assume that after double adrenalectomy, the cats have a lowered renal function. The tendency of the phenolsulphonephthalein excretion to diminish after excision of the gland also supports this idea.

The comparative experiments on the injection of urea, creatinine and sodium chloride into normal, singly adrenalectomized and doubly adrenalectomized animals indicate definitely a lowered kidney function for urea and creatinine in the experimental cats. From table 12 one sees an average excretion of 72 per cent of the injected urea and 88 per cent of the injected creatinine in normal or singly adrenalectomized animals, while the experimental cats excrete on an average only 29 per cent of the urea, and 45 per cent of the creatinine injected. These findings are confirmed by the blood analyses.

From table 11, the average difference in the urea content of the blood before and at the end of the experiment is seen to be 24 mgm. per 100 cc. and in the creatinine content, 0.3 mgm. per 100 cc. for the control animals, while in the experimental animals, the differences are for urea, 55 mgm., and creatinine, 1.7 mgm., or in the former over twice and the latter over eight times the amounts for the controls.

The chlorides are excreted in an inconstant manner under the condition of the experiments, but one notices no greater variations in the ability of the experimental animals to excrete sodium chloride than in the control. Therefore, it appears that in the case of sodium chloride, the kidneys of the adrenalectomized animals handle it equally as well as the control. However, due to the large variations in the chloride excretion we do not feel justified in emphasizing this point.



Water is excreted by the doubly adrenalectomized animals equally as well as by normal animals as shown from the injection experiments. The singly adrenalectomized animals, however, in general respond to the injection with a greater excretion of water than the normal, due undoubtedly to an interference with the branches of the splanchnic going to the kidney during the removal of the gland. The amount of urea and creatinine excreted after the injection is rather independent of the amount of water excreted, as is seen in the wide variation in the water excreted in table 12.

Histological studies have shown no definite visible changes in the kidneys of animals simply dying from adrenal insufficiency. There were, however, rather strikingly extensive fat deposits in the epithelium of the convoluted tubules in adrenalectomized cats killed during diuresis. It would seem therefore that these kidneys were more susceptible than normals to diuretic influences, though as seen above the output of solids was defective. We can not make further positive assertions concerning the significance of this picture since it is seen to a markedly less extent in normal diuretic kidneys. It may represent the effect of deleterious influences, or the effect of an effort by the kidney to overcome unfavorable conditions.

From a consideration of the evidence offered above, we feel that a functional depression of the kidney following adrenal extirpation may be considered as established.

When this renal functional depression was first demonstrated, the possibility of course at once arose that it represented the mechanism of adrenal death, that adrenal extirpation simply precipitated a renal insufficiency, with subsequent uremic termination. Our later experiments however all operate to exclude this possibility, since at the time when serious premortal symptoms, as described above, begin, the retention is as yet insufficient to explain them as uremic manifestations, in the ordinary sense of that term. Therefore, we must suppose that the renal functional depression herein described is only a part of the entire picture of adrenal insufficiency and not solely responsible for death.



The question arises as to how double adrenal extirpation causes the kidneys to become less efficient. The following possibilities have suggested themselves:

1. The renal depression may be due to circulatory disturbances characterized principally by a lowered blood pressure following adrenalectomy.

2. The interference with the nerves going to the kidney, which is unavoidable in adrenal extirpation, might be suggested as the cause of lowered kidney function.

3. Adrenal extirpation may be followed by a general diffuse lowering of cell activity in the body in which the kidney necessarily is involved.

4. The adrenal may normally neutralize some toxic substance which depresses renal activity.

5. The adrenal may normally secrete some substance which stimulates renal function.

If one of the first three explanations be correct, there would appear to be no specific relationship between the adrenals and the kidneys. On the other hand the last two possibilities would involve a more or less specific relationship in the functioning of these glands. While it might be stated at once that we feel that the evidence and data at hand are not sufficient to decide definitely as to whether there is a specific relationship, certain of the above possibilities do not appear tenable. The findings of a decreased kidney function in animals which die within a day after the complete extirpation of the adrenals can easily be attributed to the lowered blood pressure and moribund condition of the animals. The final rise of urea in the blood just before death might also be due to this cause. But in animals which survive for several days, these changes in renal activity can be demonstrated shortly after the removal of the glands. These animals are clinically in excellent condition until shortly before death. Elliott (16) has shown that in cats operated on by the interval methods, the blood pressure remains high and the reaction of the vascular system to drugs remains normal until a short time before death. Our findings have confirmed this, and animals with a definite urea retention have shown a blood

pressure within normal limits. Cat B9, which lived for seven days, on the day preceding death had a blood pressure of 115 mm. Cat B42, doubly adrenalectomized, which gave the characteristic decreased output and retention in the blood of urea and creatinine after injection had a blood pressure at the end of the experiment of 113 mm., while Cat B44, a normal animal showed at the end of an injection experiment a blood pressure of 117 mm. In general, however, as measured in the series of injection experiments, the blood pressure of adrenalectomized cats is lower than that of the control animals. Two facts however should be considered in this connection. The blood pressure was taken at the end of the injection experiments after the cats had been manipulated considerably, and we have stated above how exercise, excitement or exertion deleteriously affect these cats. Also in taking blood pressures without an anaesthetic, slight struggling of the animals or excitement affects the pressure considerably. However, the animal B42, noted above, did not struggle at the time the pressure measurement of 113 mm. was taken. Therefore, in view of the fact that the typical changes may occur with a normal pressure, and of Elliott's blood pressure findings, we feel that it is difficult to explain the findings on circulatory changes alone.

The injury to the nerves going to the kidney in removing the adrenal, certainly does not explain the functional changes in that organ. The experiments quoted above, in which cats with one adrenal removed and the splanchnic on the other side sectioned, showed no rise of urea in the blood, tends to disprove this hypothesis. Again Quinby (17) has shown that the kidney removed from the body and deprived of its nerves when reimplanted has first a period of hyperactivity and then a normal function. So the changes from this cause are just the reverse of those described in this paper.

A general decrease in all bodily functions probably takes place in adrenalectomized animals when the prostration and low blood pressure develop. Whipple (18) has shown a decrease in liver function as measured by the phenoltetrachlorophthalein test in adrenalectomized dogs, while Friedman (19)

found gastric ulcers as a rather constant condition in dogs and rabbits dying of adrenal extirpation. An influence of the adrenals on the pancreas has been postulated, (20) but Mann and Drips (21) have shown this to be incident to the low blood pressure and moribund condition of the animals. In the earlier days after adrenalectomy in our cats, one cannot assume any such moribund condition. Moreover, there is no decrease of the protein catabolism which one would expect if bodily activity were lowered in general.

It has been frequently suggested that the adrenals function as detoxifying agents and that after adrenal extirpation death is brought about by toxic agents. Gautrelet and Thomas (22) found that the blood of animals deprived of their adrenals was toxic when injected into normal animals causing a marked lowering of blood pressure, but Gradinescu (23) in a number of experiments could not confirm this finding.

The excretion of some substance by the adrenals which is necessary for normal kidney function, and the consequent inter-relationship of the two glands serves as a very probable explanation of the results which are presented in this paper. Should it be found possible to prevent the renal changes in animals deprived of their adrenals by injection of adrenal extracts, it would support this hypothesis. However, a negative result in this direction would not appear conclusive, as one can not by injection of a gland extract, reproduce the essentials of the functioning of that gland in the animal organism, and it is known that adrenal therapy does not prevent death in animals deprived of these glands. We have conducted a few experiments along these lines which have been inconclusive, but shall continue them in the near future.

#### SUMMARY

1. Cats, from which both adrenal glands have been completely removed by the interval method and precautions employed by Elliott, have survived from one to seven days.

2. The urea concentration in the blood rises after complete removal of the adrenals to about twice the normal value and



remains approximately stationary at this level until shortly before death when it again rises.

3. The phenolsulphonephthalein excretion shows a tendency to diminish after adrenalectomy.

4. Cats with both adrenals removed excrete much less urea and creatinine in the urine after an injection of these substances than normal or singly adrenalectomized animals.

5. The kidneys of adrenalectomized animals show no noticeable histological change from the normal, but those of adrenalectomized animals which have received an injection of urea, creatinine and sodium chloride show a striking change from the control animals.

6. The nitrogen excretion in the urine of adrenalectomized cats is slightly diminished after the operation; the diminution being accounted for by the retention of nitrogen products in the organism. There is hence no marked change in protein catabolism.

7. The above facts, which we have demonstrated, indicate a marked lowering of kidney efficiency in adrenalectomized cats. This may occur with a normal blood pressure, and when the animals are in excellent physical condition.

8. The bearing of these facts on the inter-relationship of the adrenals and kidneys is discussed, and the excretion of some substance by the adrenals which is necessary for the maintenance of normal kidney function serves as a probable explanation of the results which we have obtained.

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# THE PHARMACOLOGY OF THE VAS DEFERENS

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## I. INTRODUCTORY

The vas deferens does not appear to have been investigated by pharmacologists. Indeed, an examination of the literature relating to the organ has disclosed references to the action of but two drugs, atropine and nicotine, these two having been examined by Langley and Anderson (1) in the course of physiological investigations.

As is well known, the vas deferens possesses a very thick wall consisting of three layers of smooth muscle, an outer longitudinal, a middle circular, and an inner longitudinal. These muscular coats are innervated, according to Langley and Anderson, by motor sympathetic nerves, which arise as post ganglionic fibers from the inferior mesenteric ganglion or from accessory ganglia lying in the course of the hypogastrics. The above authors could find no evidence of a para-sympathetic nerve supply and anatomists do not describe ganglion cells in the wall of the organ.

Schaefer (2) and Marshall (3) in their articles on the physiology of reproduction state that the vas deferens undergoes peristaltic contractions during coitus. There is, however, a difference of opinion among physiologists as to whether the movements of the organ are true peristaltic ones or simple contractions, the effect of electrical stimulation of the organ in situ being given the former interpretation by Fick (4) and Budge (5) and the latter by Loeb (6) and Nagel (7). As none of the investigators mention movements in the vas deferens when not under the influence of stimuli (heat, cold, electricity, etc.), the inference is that they found it to be quiescent.

## II. METHOD OF STUDY

In this paper are reported the results obtained with the excised vasa deferentia of sheep, dogs, rabbits, rats, and guinea pigs. The drugs used were epinephrine, ergot, hydrastinine, nicotine, pilocarpine, pituitary extract, and barium chloride.

In the case of the dogs, rabbits, rats and guinea pigs, the organ was removed under complete ether or chloroform anaesthesia; in that of the sheep, it was obtained from the carcass of a freshly slaughtered lamb at the city abattoir. There was an interval of about two hours elapsing between the death of the sheep and the beginning of the experiment, during which time the organ was kept in a dry glass-stoppered bottle. In most instances where laboratory animals were used, the organ was transferred immediately from the sleeping animal to the oxygenated bath.

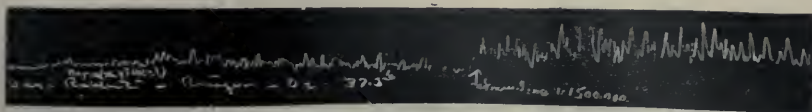
The suspension method was the one employed in all my experiments. The procedure was in brief as follows:

A piece of the vas deferens, stripped of all surrounding tissue and measuring 2 to 5 cm. in length, was attached by one extremity to a stationary hook and by the other with a silk thread to a lever, the movements of which were recorded on a slowly moving drum. The tissue with its attached hook was immersed in an oxygenated bath of Tyrode's (8) or Ringer's solution, the bath being connected with a reservoir for supplying fresh fluid. The bath, the reservoir, and all their connections were kept submerged in a wash boiler equipped with a heat regulator of sufficient delicacy to maintain the temperature at 37-38°C.<sup>1</sup>

## III. DATA FROM SUSPENDED LONGITUDINAL SECTIONS

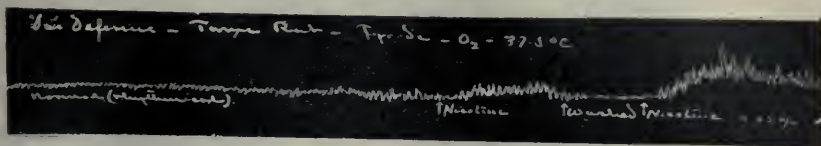
1. *Normal.* The freshly excised vasa deferentia of the rat and the rabbit exhibited slow rhythmic contractions on being suspended in oxygenated Ringer's and Tyrode's solutions. These contractions were not observed in the majority of instances, and never in the case of the dog, the guinea pig, and the sheep. That they were not shown on the records in all cases, may have been due to technical errors.

<sup>1</sup> For a detailed description of the apparatus employed, the reader is referred to Roth's article on the standardization of pituitary extract (9).



TRACING 1

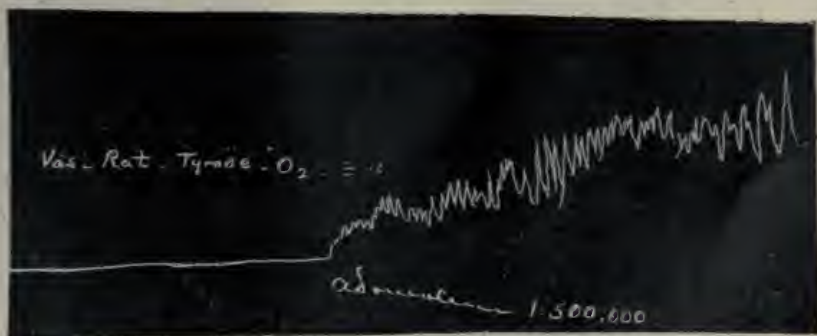
Vas deferens of rabbit, suspended in Ringer's solution. Normal rhythmic contractions are shown, and also the influence of adrenaline.



TRACING 2

Vas deferens of rat, suspended in Tyrode's solution. Normal rhythmic contractions are shown, and also the influence of nicotine.

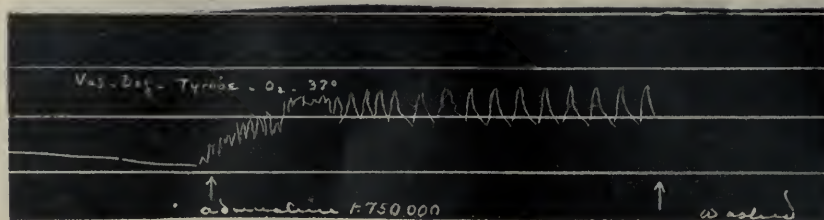
2. *Epinephrine*. Epinephrine (Adrenaline, P. D. Co.) was tried on the vasa deferentia of all the animals of my series (dogs, sheep, guinea pigs, rats and rabbits). In every case, its application was followed by a marked increase in tone in both the rhythmically active and in the quiescent organ. The force and extent of the contractions were increased in the former; rhythmic contractions were produced in the latter, subsequent to the increase in tone (tracings 1, 3, 4, 5, 6 and 7).



TRACING 3

Vas deferens of rat, suspended in Tyrode's solution. The effect of adrenaline on the quiescent organ is shown.



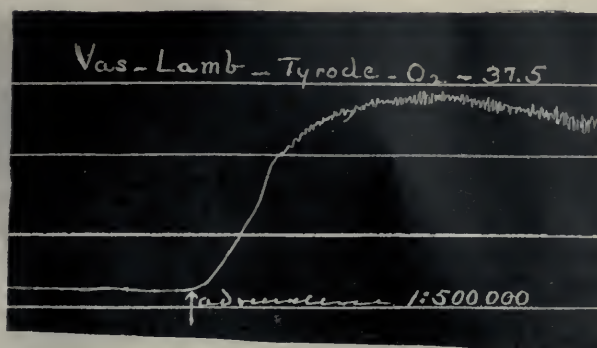


TRACING 4

Vas deferens of dog, suspended in Tyrode's solution. The effect of adrenaline is shown.

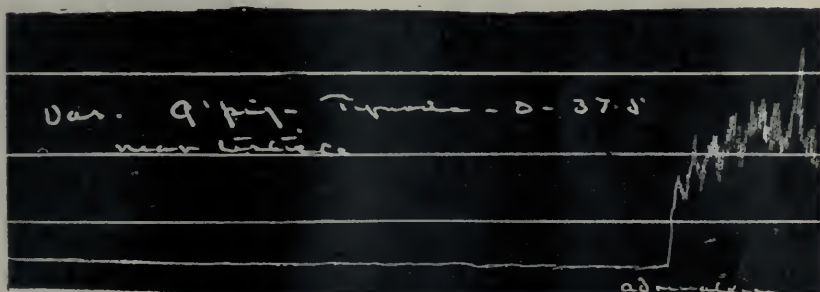
The following points disclosed in the action of epinephrine should be noted: (1) The latent period was very short. (2) Both tone and rhythm were affected. (3) The increase in tone always preceded the change in rhythmicity. (4) The effects persisted as long as the tissue was in contact with epinephrine and promptly disappeared when the bath was changed. (5) Epinephrine did not seem to injure the organ; repeated application, in some experiments, produced no appreciable qualitative or quantitative alteration in the reaction. (6) The effect was quantitative in respect to tone changes. (7) The freshly excised vas deferens and that which had been kept for two to four hours exposed to the air or twenty-four hours in Tyrode's solution at body temperature reacted qualitatively alike. (8) The effects produced by epinephrine were in accord with Langley and Anderson's conclusion that this organ has a motor sympathetic innervation.

3. *Ergot*. The fluid extract of ergot (alcohol not removed) was tried on the vasa deferentia of the sheep and the rat. The effects produced were similar to those following the application of epinephrine. The latent period was, however, somewhat longer (compare tracing 8 with 5). According to the work of Dale (10), the action of this drug, too, is in harmony with the possession of a motor sympathetic innervation.



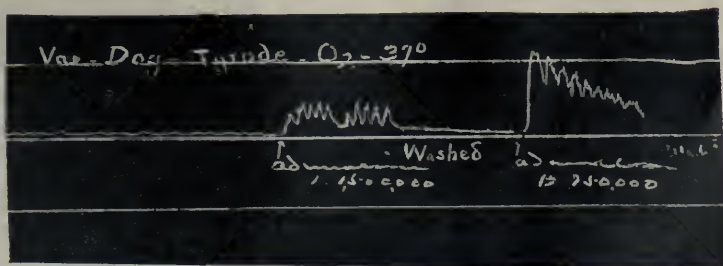
TRACING 5

Vas deferens of sheep, suspended in Tyrode's solution. The effect of adrenaline is shown.



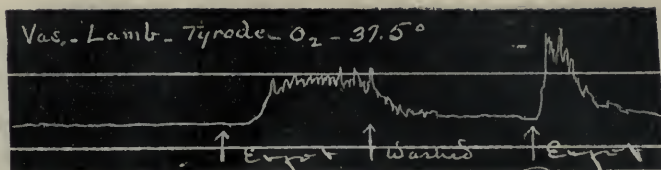
TRACING 6

Vas deferens of guinea pig, suspended in Tyrode's solution. The effect of adrenaline is shown.



TRACING 7

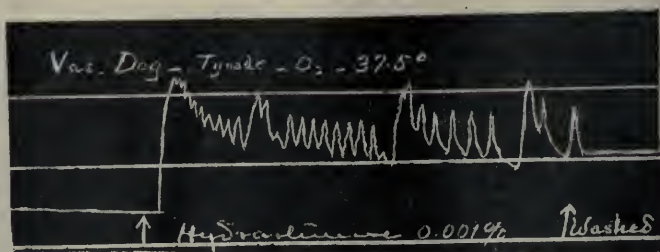
Vas deferens of dog, suspended in Tyrode's solution. The quantitative effect of adrenaline is shown.



TRACING 8

Vas deferens of sheep, suspended in Tyrode's solution. The effect of fluid extract of ergot is shown.

4. *Hydrastinine*. Hydrastinine was tried on the vasa deferentia of the dog and the rat. The effect was like that produced by epinephrine. Cotarnine and fluid extract of hydrastis acted similarly.

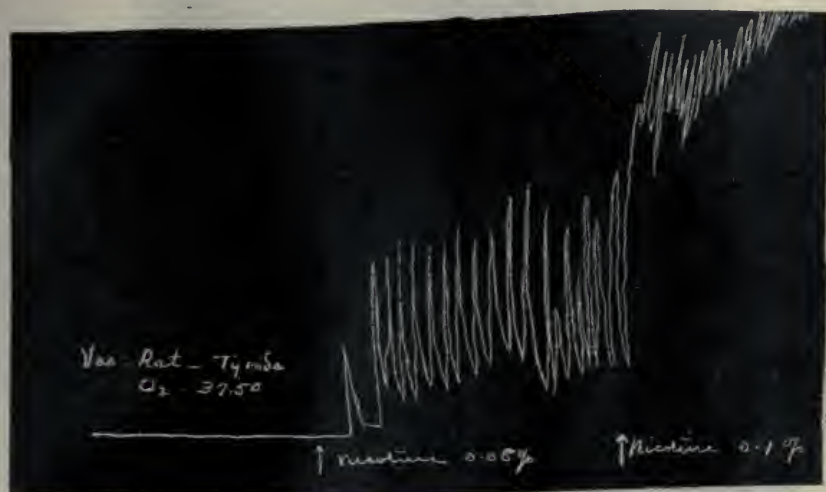


TRACING 9

Vas deferens of dog, suspended in Tyrode's solution. The effect of hydrastinine is shown.

5. *Nicotine*. Nicotine was tried on the vasa deferentia of rats, guinea pigs, and rabbits. It increased the force of the contractions and raised the tone in the rhythmically acting organ and produced rhythmic contractions and increased the tone in the quiescent. The effect on rhythm preceded that on tone which is contrary to the usual behavior of smooth muscle. A very high concentration of nicotine was necessary and after its application epinephrine was quantitatively less effective than previously, a detail in which the vas deferens resembles the ureter as shown in Macht's experiments (11). The reactions to nicotine would seem to indicate the presence of ganglion cells in the wall of the organ.

6. *Pilocarpine*. Pilocarpine was tried on the vasa deferentia of the rat and the rabbit. In strong concentrations, it increased the tone and produced rhythmic contractions in the quiescent organ. The effect on tone was marked in both species of animals; that on rhythm, less marked in the case of the rabbit. The duration of the effect was brief, the concentration of the drug necessary to produce stimulation probably being near that to produce depression (compare Macht's reactions of the ureter



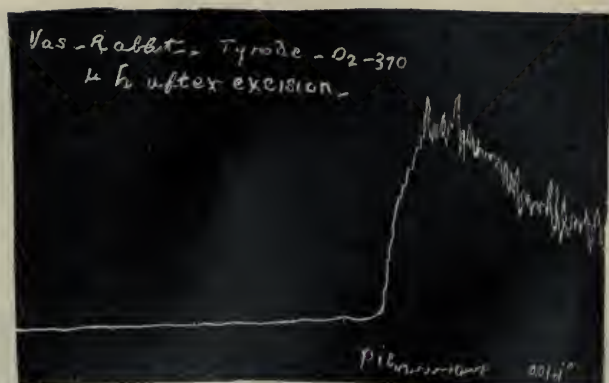
TRACING 10

Vas deferens of rat, suspended in Tyrode's solution. The effect of nicotine on the quiescent organ is shown.

(12). The positive results with this drug would, on the basis of the accepted view of its point of action, indicate the presence of post-ganglionic para-sympathetic motor fibers, which is contrary to the conclusions of Langley and Anderson.

7. *Pituitary extract*. The pituitary liquid of Armour and the pituitrin of Parke, Davis and Company produced no effect on the quiescent vasa deferentia of any of the animals of my series. In view of its marked action on the internal generative organs of the female, it is remarkable that this drug should fail to produce results on the vas deferens.

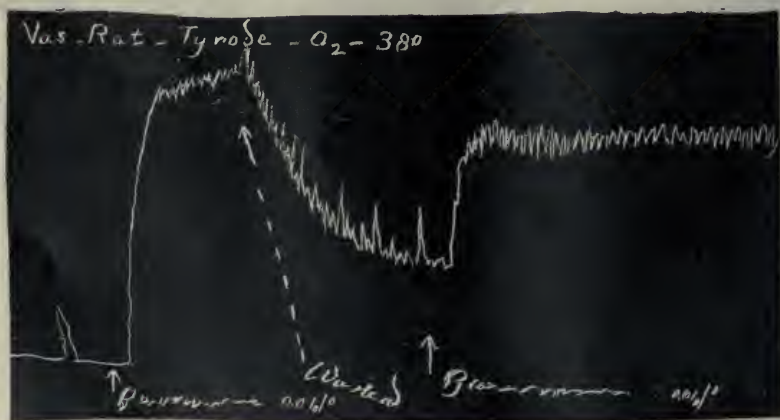




TRACING 11

Vas deferens of rabbit, suspended in Tyrode's solution. The effect of pilocarpine is shown.

8. *Barium chloride.* Barium chloride was tried on the vasa deferentia of the rat, the rabbit, and the dog. The effect was like that produced by epinephrine, though the latent period was somewhat longer and changing the bath did not at once restore the resting condition. High concentrations did not seem to injure the tissue, and under the influence of the drug rhythmic activity could be maintained for hours.



TRACING 12

Vas deferens of rat, suspended in Tyrode's solution. The effect of barium chloride is shown.

## IV. SUMMARY

1. The freshly excised vasa deferentia of the rabbit and the rat exhibit rhythmic contractions when suspended in oxygenated Tyrode's or Ringer's solution at body temperature.

2. The vasa deferentia of dogs, rabbits, rats, guinea pigs, and sheep exhibit increased tone and rhythmic contractions upon application of epinephrine, ergot, hydrastinine, pilocarpine, nicotine, and barium chloride. All parts of the organ react essentially alike.

3. Pituitary extract produced no effect on the quiescent vasa deferentia of any of the animals examined.

4. The vas deferens is a very responsive and very resistant organ and it may prove of value in the physiological standardization of certain drugs.

I already have under way further investigations of the reactions of the vas deferens, and of other portions of the male generative tract. These will be reported at an early date.

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